

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>5</sup> :</b> C07K 7/20, 7/14, 7/12 C07K 7/04, A61K 37/43, 37/02 G01N 33/68	<b>A2</b>	<b>(11) International Publication Number:</b> WO 93/03058 <b>(43) International Publication Date:</b> 18 February 1993 (18.02.93)
<b>(21) International Application Number:</b> PCT/CA92/00281 <b>(22) International Filing Date:</b> 2 July 1992 (02.07.92)  <b>(30) Priority data:</b> 721,324 1 July 1991 (01.07.91) US  <b>(71) Applicant:</b> UNIVERSITY TECHNOLOGIES INTERNATIONAL INC. [CA/CA]; HM 382, 3310 Hospital Drive N.W., Calgary, Alberta T2N 4N1 (CA).  <b>(72) Inventors:</b> MOORE, Graham, J. ; 1762 - 1st Ave., Calgary, Alberta T2N 0B1 (CA). HABIBI, Hamid, R. ; 957 Ranchview Crescent, N.W., Calgary, Alberta T3G 1A4 (CA). MATSOUKAS, John, M. ; 29 Saint Dionissios, GR-262 23 Patras (GR).		<b>(74) Agent:</b> MITCHELL, Richard, J.; Marks & Clerk, 55 Metcalfe Street, Suite 1380, P.O. Box 957, Station B, Ottawa, Ontario K1P 5S7 (CA).  <b>(81) Designated States:</b> CA, JP, NO, RU, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> NON-DESENSITIZING ANALOGS OF GnRH AND OTHER BIOLOGICALLY ACTIVE LIGANDS  <b>(57) Abstract</b>  The present invention is directed to novel non-desensitizing analogs of a biologically active ligand having at least one 6-membered ring or 5-membered ring and a method for selecting non-desensitizing analogs of a biologically active ligand having at least one 6-membered ring or 5-membered ring. Additionally, the present invention is directed to a novel method for treating a patient in a non-desensitizing fashion with a non-desensitizing analog of a biologically active ligand having at least one 6-membered ring or 5-membered ring. Moreover, the present invention is directed to a novel method for making new and useful non-desensitizing analogs of GnRH and opiate peptides as well as the novel non-desensitizing compounds and compositions prepared thereby.		

BEST AVAILABLE COPY

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	MN	Mongolia
AU	Australia	FR	France	MR	Mauritania
BB	Barbados	GA	Gabon	MW	Malawi
BE	Belgium	GB	United Kingdom	NL	Netherlands
BF	Burkina Faso	GN	Guinea	NO	Norway
BG	Bulgaria	GR	Greece	NZ	New Zealand
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IE	Ireland	PT	Portugal
CA	Canada	IT	Italy	RO	Romania
CF	Central African Republic	JP	Japan	RU	Russian Federation
CG	Congo	KP	Democratic People's Republic of Korea	SD	Sudan
CH	Switzerland	KR	Republic of Korea	SE	Sweden
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovak Republic
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CS	Czechoslovakia	LU	Luxembourg	SU	Soviet Union
CZ	Czech Republic	MC	Monaco	TD	Chad
DE	Germany	MG	Madagascar	TG	Togo
DK	Denmark	ML	Mali	UA	Ukraine
ES	Spain			US	United States of America

-1-

**NON-DESENSITIZING ANALOGS OF GnRH  
AND OTHER BIOLOGICALLY ACTIVE LIGANDS**

**BACKGROUND OF THE INVENTION**

**Field of the Invention**

5           The present invention is directed to novel  
non-desensitizing analogs of a biologically active  
ligand containing at least one 6-membered aromatic ring  
or 5-membered ring and a method for selecting non-  
desensitizing analogs of a biologically active ligand  
10   containing at least one 6-membered aromatic ring or 5-  
membered ring. Additionally, the present invention is  
directed to a novel method for treating a patient in a  
non-desensitizing fashion with a non-desensitizing  
analog of a biologically active ligand containing at  
15   least one 6-membered aromatic ring or 5-membered ring.  
Moreover, the present invention is directed to a novel  
method for making new and useful non-desensitizing  
analogues of GnRH and opiate peptides as well as the  
novel non-desensitizing compounds prepared thereby.

20           **State of the Art**

          In living organisms, the interaction of a  
biologically active ligand with a receptor to produce a  
biological response is often accompanied by  
desensitization of the receptor, such that a subsequent  
25   interaction of the ligand with the receptor results in  
attenuation of the subsequent biological response to  
the ligand. Prior to the present invention, however,  
very little was understood about the causes and effects

-2-

surrounding the desensitization process or how such detrimental desensitization could be avoided. Thus, the primary focus up to this point has been attempts to develop agonistic and antagonistic analogs of  
5 biologically active ligands with little or no regard to their potential desensitizing effects.

For example, Gonadotropin-Releasing Hormone (GnRH) is one such biologically active compound which has been modified to produce both agonistic and  
10 antagonistic analogs thereof. GnRH stimulates the release of gonadotropins through interaction with membrane-associated high affinity receptors on the pituitary gonadotropes. Subsequently, these gonadotropins act on the gonads to stimulate the  
15 synthesis of steroid sex hormones. The pulsatile release of GnRH, and thereby the release of gonadotropins, controls the reproductive cycle in domestic animals and humans.

Acute doses of GnRH agonists administered in  
20 pulsatile fashion can increase the levels of Luteinizing Hormone (LH) and steroid sex hormones in both animals and humans. Paradoxically, acute or chronic doses of these agonists can suppress the levels of LH and steroid hormones. Additionally, the effect  
25 of multiple doses of GnRH agonists can be to suppress estrogen formation in the female and suppress testosterone formation in the male. Accordingly, knowledge of the molecular process by which desensitization occurs would be extremely useful in the  
30 design of analogs of biologically active ligands in which said receptor desensitization effects are reduced.

-3-

Thus, it is an object of the present invention to provide novel non-desensitizing analogs of a biologically active ligand containing at least one 6-membered aromatic ring or 5-membered ring.

5 Yet another object of the present invention is to provide a novel method for selecting non-desensitizing analogs of a biologically active ligand which contain at least one 6-membered aromatic ring or 5-membered ring.

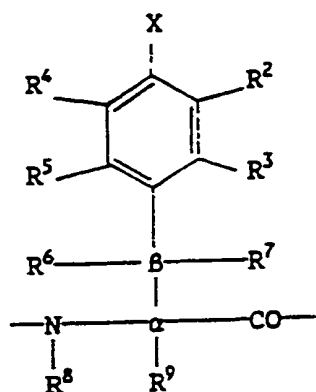
10 Still another object of the present invention is to provide a novel method for treating a patient in a non-desensitizing fashion with a non-desensitizing analog of a biologically active ligand containing at least one 6-membered aromatic ring or 5-membered ring.

15 A further object of the present invention is to provide a novel method for producing new and useful analogs of GnRH and opiate peptides as well as the novel analogs produced thereby.

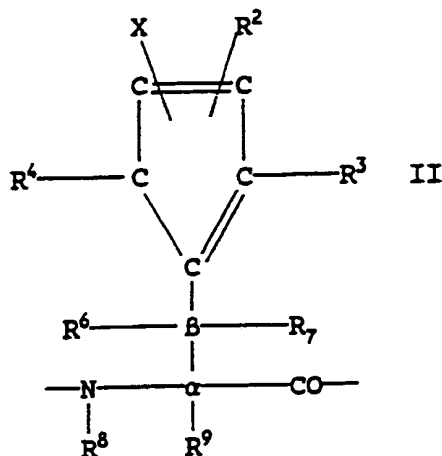
#### SUMMARY OF THE INVENTION

20 In accordance with the foregoing objectives, there is provided a method for selecting non-desensitizing analogs of a biologically active ligand containing at least one 6-membered aromatic ring or 5-membered ring. The method comprises selecting an  
25 analog of a biologically active ligand containing a 6-membered aromatic ring according to Formula I or selecting a biologically active ligand containing a 5-membered ring according to Formula II:

-4-



I



II

- In Formulas I and II, above, X is selected from H, R<sup>1</sup>, -OR<sup>1</sup>, halide, -CN, -CHO, C(halide)<sub>3</sub>, -alk-OH, -alk-OR<sup>1</sup>, -alk-CO<sub>2</sub>H, -alk-CO<sub>2</sub>R<sup>1</sup>, -alk-SH, -alk-SR<sup>1</sup>, -alk-CONH<sub>2</sub>, -CO<sub>2</sub>H, -CO<sub>2</sub>R<sup>1</sup>, -COR<sup>1</sup>, -OCONH<sub>2</sub>, -OCH<sub>2</sub>OH, -OCH<sub>2</sub>OR<sup>1</sup>, -OCOR<sup>1</sup>, -N<sub>3</sub>, -N<sub>2</sub>, -NHCOR<sup>1</sup>, -NO<sub>2</sub>, -NH<sub>2</sub>, -NHR<sup>1</sup>, -NR<sup>1</sup><sub>2</sub>, -SO<sub>3</sub>H, -SO<sub>2</sub>R<sup>1</sup>, -SCOR<sup>1</sup>, -NCS, -SCSR<sup>1</sup>, -SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NHR<sup>1</sup>, -SO<sub>2</sub>NR<sup>1</sup><sub>2</sub>, -SO<sub>4</sub>H, -PO<sub>3</sub>H, -PO<sub>4</sub>H<sub>2</sub>, -SH, -SR<sup>1</sup>, -N=N-R<sup>1</sup>, =O, =NH, =N-R<sup>1</sup>, =N-OH, =N-OR<sup>1</sup>, -CONH<sub>2</sub>, -CONHR<sup>1</sup>, -CONR<sup>1</sup><sub>2</sub>, -NH-SO<sub>2</sub>R<sup>1</sup>, -SiR<sup>1</sup><sub>3</sub>, tetrazole, imidazole, or substituted or unsubstituted phenyl, -alkylphenyl, -O-phenyl, -O-alkylphenyl, -O-benzyloxy-carbonyl. Additionally, R<sup>1</sup> is selected from alkyl of 1-7 carbon atoms, alkenyl or alkynyl of 2-7 carbon atoms or cycloalkyl of 3-7 carbon atoms, optionally halogenated at one or more hydrogen.
- Likewise, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup> and R<sup>9</sup> are groups selected from X or -OH. In the above, α is selected from P, N, S, or C being in either the L- or D-configuration and β can be deleted or extended by 1-2 carbon atoms, substituted or unsubstituted. Moreover, the nitrogen atom attached to α can be substituted or unsubstituted C, S, O or P and the carbon atom attached to α and oxygen can be substituted or unsubstituted N, S, O, P or C. The aromatic ring of Formula I or the ring of Formula II can contain 0-4 N, S or O atoms.

-5-

Likewise, the aromatic ring of Formula I or ring of Formula II can be fused with a 6-membered aromatic ring which can contain 0-4 N, S or O atoms and which can be substituted in the same manner as the aromatic ring of Formula I. Also included within Formulas I and II are D-tyrosine and D-histidine.

Also provided is a method of treating a patient in a non-desensitizing fashion with a non-desensitizing analog of a biologically active ligand containing at least one 6-membered aromatic ring or 5-membered ring. The method comprises selecting an analog of a biologically active ligand containing a 6-membered aromatic ring according to Formula I, as defined above, or selecting a biologically active ligand containing a 5-membered ring according to Formula II, as defined above. The next step in the method comprises administering to a patient in need thereof a composition comprising a pharmaceutically effective amount of the selected analog together with a pharmaceutically acceptable carrier.

Also provided are new, non-desensitizing analogs of a biologically active ligand containing at least one 6-membered aromatic ring according to Formula I or 5-membered ring according to Formula II, as defined above, but excluding the following compounds:

- (1) antagonist analogs obtained by substituting D-amino acids at positions 1, 2 or 3, and analogs which have position 2 deleted;
- (2) the following agonist analogs based on human GnRH:

-6-

- (A) [D-Phe<sup>6</sup>]GnRH in which the hydroxyl group of tyrosine is protected with benzyl, acetyl, tosyl, benzoyl, t-butyl, tetrahydropyran-2-yl, trityl, 2,4-dichlorobenzyl, benzyloxycarbonyl or 2-bromobenzyloxycarbonyl;
- (B) [Phe<sup>5</sup>]GnRH, [Ala<sup>4</sup>Phe<sup>5</sup>]GnRH and [Phe(N<sub>3</sub>)<sup>5</sup>]GnRH;
- (C) [Des<sup>10</sup>Phe<sup>5</sup>-D-Ala<sup>6</sup>ProNHR<sup>9</sup>]GnRH in which position 2 is His, Tyr, Trp or Phe(4'-NH<sub>2</sub>), position 7 is Leu, Ile or Nle, and R is Et, Pr, CH<sub>2</sub>CH<sub>2</sub>OH or CHMe<sub>2</sub>;
- (D) [Des<sup>10</sup>X<sup>5</sup>Pro-B<sup>9</sup>]GnRH in which position 3 is Trp, Phe or 3(1-naphthylalanine); X is His, Phe or Phe(F<sub>5</sub>); position 6 is D-Ala(Y) wherein Y is naphthyl, anthryl, fluorenyl, phenanthryl, biphenyl, benzhydryl, phenyl or cyclohexyl with 3 or more alkyl groups, perhydronaphthyl, adamantyl, perhydrobenzhydryl, phenyl, cyclohexyl, dicyclohexyl, heterocycle aryl, optionally N-alkylated alkylamine or cycloalkylamine, or NHCH[(CH<sub>2</sub>)<sub>n</sub>NHC(=NR<sub>2</sub>)R<sub>1</sub>]CO wherein: n is 1-5; R<sub>1</sub> is alkyl or NRR<sub>3</sub>, wherein R is H or alkyl and R<sub>3</sub> is H, alkyl, fluoroalkyl, cycloalkyl, phenyl, benzyl, alkyl-morpholino or (CH<sub>2</sub>)<sub>n</sub>(R<sub>4</sub>)<sub>2</sub>, wherein n is 1-5 and R<sub>4</sub> is alkyl; R<sub>2</sub> is H or R<sub>3</sub>; and wherein C=NR<sub>2</sub>R<sub>1</sub> can be a ring; position 7 is Leu, Ile, MeLeu or Trp; position 8 is Arg, Gln, Tyr or Leu; B is selected from GlyNH<sub>2</sub>, NHB<sub>2</sub> wherein B<sub>2</sub> is alkyl, cycloalkyl or haloalkyl, and NHCONHB<sub>3</sub>, wherein B<sub>3</sub> is H or alkyl;
- (E) analogs containing a gamma (γ) lactam in the 6-7 position, in which position 1 is Pyr, N-acetyl, N-Pyr-imino acid, or (C<sub>3-7</sub> cycloalkyl)acyl; positions 2 and 3 are aliphatic or aromatic amino acid; position 4 is Ser, Thr or Ala; position 5 is an aromatic amino acid His, Trp or Phe; position 8 is an amino acid with a basic sidechain; position 9 is an imino acid or aliphatic amino acid; position 10 is GlyNH<sub>2</sub>, AlaNH<sub>2</sub>, NHet, NHPr, NHCH<sub>2</sub>CH<sub>2</sub>OH; and



-7-

(F) analogs in which position 5 is Phe, Tyr(Me), N-alkyl-Phe, N-alkyl-Tyr(Me) or N-alkyl-Tyr(Et), wherein the nitrogen atom of at least one of the amide bonds is alkylated.

5 Also provided is a novel method for synthesizing new and useful analogs of GnRH and opiate peptides as well as the novel analogs produced thereby.

Also provided are the distinctly novel non-desensitizing analogs having the Formula III:

10  $X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}$  III.

In Formula III, above,  $X_1$  is L- or D-Pyr, N-acyl-amino acid,  $\alpha$ -C-alkyl-Pyr, or N-acyl- $\alpha$ -C-alkyl-amino acid;  $X_2$ ,  $X_3$ ,  $X_4$ ,  $X_6$ ,  $X_7$  and  $X_8$  are independently selected from natural, synthetic, protected or  $\alpha$ -C-alkyl- amino  
15 acids, or  $X_6-X_7$  optionally contains a  $\gamma$ -lactam;  $X_9$  is an imino acid or  $\alpha$ -C-alkyl-imino acid;  $X_{10}$  is -N(H)R wherein R is GlyNH<sub>2</sub>, azaGlyNH<sub>2</sub>, alkyl or alkenyl, cycloalkyl, haloalkyl, hydroxyalkyl or aryl; and  $X_5$  is selected from Formulas I or II, as defined above. The  
20 compounds excluded from the compounds according to Formulas I and II are likewise excluded from the compounds according to Formula III

Also provided are novel agonist analogs according to Formula III, wherein  $X_1$  is Pyr, N-acyl-  
25 amino acid,  $\alpha$ -C-alkyl-Pyr, N-acyl- $\alpha$ -C-alkyl amino acid;  $X_2$  and  $X_3$  are aromatic L-amino acids;  $X_4$ ,  $X_7$  and  $X_8$  are independently natural, synthetic, protected or  $\alpha$ -C-alkyl- amino acids;  $X_6$  is a natural, synthetic or protected D-amino acid, Gly,  $\alpha$ -C-alkyl-amino acid; or  
30  $X_6-X_7$  optionally contains a  $\gamma$ -lactam;  $X_9$  is imino acid or  $\alpha$ -C-alkyl-imino acid;  $X_{10}$  is -N(H)R wherein R is GlyNH<sub>2</sub>, azaGlyNH<sub>2</sub>, alkyl, alkenyl, cycloalkyl, haloalkyl, hydroxyalkyl or aryl; and  $X_5$  is according to Formulas I

-8-

or II. The compounds excluded from the compounds according to Formulas I and II are likewise excluded from these compounds according to Formula III.

#### DETAILED DESCRIPTION OF THE INVENTION

5           Although biologically active ligands have been the subject of numerous studies in which there have been a multitude of agonistic and antagonistic analogs created, there has been no successful attempts at developing criteria by which one can select  
10 agonistic and antagonistic analogs of biologically active ligands which are non-desensitizing in nature. For this reason, the discovery by the present inventors is potentially very far reaching in scope. That is, the present inventors have discovered that by  
15 selectively modifying a biologically active ligand containing at least one 6-membered aromatic ring or 5-membered ring in the manner according to the present invention, that it is possible to produce analogs of a biologically active ligand which are non-desensitizing  
20 in nature. It is to be understood that the term "non-desensitizing" describes a biologically active ligand with at least partially reduced desensitizing properties.

          As used herein, the term "biologically active  
25 ligand" refers to a molecule which binds to a biologically active receptor molecule and which directly or indirectly affects the activity of the receptor molecule. The binding of such ligands to the receptor (acceptor) molecule is accordingly a necessary  
30 precondition for initiating, terminating, altering or preventing the biological activity in the receptor molecule. Any ligand which effects the biological activity of the receptor molecule is said to be a

-9-

biologically active ligand. The biologically active ligand can be a substrate, an agonist, an antagonist, an activator, an inhibitor, etc. Examples of biologically active ligands according to the present invention include GnRH, also known as Luteinizing Hormone-Releasing Hormone (LH-RH), and Angiotensin (II or III), and the like. Additional examples of biologically active ligands are well documented in the art.

Moreover, the biologically active ligand can be either peptidic or non-peptidic in nature. Such ligands can be indigenous to the organism where the biologically active receptor is found. When the ligand is one which is naturally occurring in that organism, then that ligand is referred to as a naturally occurring biologically active ligand. On the other hand, the biologically active ligands can be synthetic molecules which are complementary to the biologically active receptor and which affect the biological activity of the receptor. Thus, any molecule which is complementary to a biologically active receptor and which affects the biological activity of the receptor, is a biologically active ligand.

The term "ligand" according to the present invention refers to any organic compound for which a receptor naturally exists or can be prepared. The term "receptor" according to the present invention refers to a molecule which binds the ligand.

When binding of the biologically active ligand to the biologically active receptor and the activation of the active site results in an alteration of the biological activity of the receptor, e.g., initiates, increases, decreases or terminates the

-10-

biological activity of the receptor, the ligand is said to directly affect the activity of the receptor. On the other hand, a biologically active ligand indirectly affects the activity of the biologically active  
5 receptor when the binding of the ligand to the receptor results in an inability to activate the receptor.

Activation of the active site of the naturally occurring biologically active ligand/receptor complex is generally accomplished by some sort of  
10 chemical interaction between the ligand and the receptor. When the chemical interaction involves the transfer of charge from one residue to another wherein one of the residues is either a phenol or phenolate  
15 interaction. Such charge-transfer interactions are believed to result in the alteration of the structure of the ligand or ligand/receptor complex. Because such charge-transfer interactions can now be detected by the  
20 techniques employed in the present invention, it is now possible to incorporate such interactions into the model created for the naturally occurring biologically active ligand and to create agonists and antagonists which have reduced desensitizing properties at the complementary receptor.

25 Any biologically active ligand containing a phenolic group, such as tyrosine, may utilize the phenolic group to desensitize a complementary receptor. Methods for determining biologically active phenolate ligands using spectroscopic techniques have been  
30 described in detail in U.S. Ser No. 07/458,926 filed December 29, 1989, which is incorporated herein by reference.

-11-

In one aspect of the method according to the present invention, the presence of a phenolate species in a biologically active ligand is ascertained by measuring the fluorescence lifetime due to said  
5 phenolate species in a suitable environment such as propylene glycol. The presence of long lifetime phenolate (tyrosinate) fluorescence is diagnostic of the occurrence of the active phenolate species which may cause receptor desensitization. The long  
10 fluorescence lifetime is produced by intramolecular interactions within the biologically active ligand when the ligand is dissolved in a receptor-simulating solvent such as propylene glycol.

In another method aspect, the lability of the  
15 phenolic OH proton may be determined by NMR spectroscopy of the biologically active ligand in a suitable solvent such as DMSO. The absence of a NMR signal characteristic of the phenolic OH proton, at or about 9.2 ppm, is also an indication of the presence of  
20 a phenolate species which may be involved in a receptor desensitization process. Furthermore, and as described in the present disclosure, modification or substitution of the phenolic OH group of a biologically active ligand provides a method for establishing the  
25 involvement of the phenolic OH group in a receptor desensitizing process, particularly when such modification results in a ligand with reduced receptor desensitizing properties, as exemplified herein for peptides based on Angiotensin II and GnRH.

30

The term "agonist" according to the present invention refers to a biologically active ligand which binds to its complementary biologically active receptor and activates the latter either to cause a biological  
35 response in the receptor or to enhance pre-existing

-12-

biological activity of the receptor. The agonist can be the naturally occurring biologically active ligand or it can be a synthetic molecule which can also activate the receptor. For example, it is known in the art that Angiotensin II acts as an agonist for its complementary receptor, the Angiotensin II receptor. Other examples of agonists for the Angiotensin II receptor include [Sar<sub>1</sub>] Angiotensin II and the like. A common characteristic of all ligands in this invention is that the charge-transfer interaction in the ligand which is necessary to desensitize the biologically active receptor is compromised. That is to say that the charge-transfer interaction is essentially inoperable in the ligand.

The term "antagonist" refers to a biologically active ligand which binds to its complementary biologically active receptor and either prevents the activation of the latter or deactivates the latter so as to either prevent or diminish the biological activity of the receptor. For example, it is known in the art that the non-peptides 2-n-butyl-1-[4-carboxybenzyl]-4-chloroimidazole-5-acetic acid) and (methyl 2-n-butyl-1-[4-(2-carboxybenzamido)-benzyl]-4-chloroimidazole-5-acetate) sodium salt act as antagonists of the Angiotensin II receptor. Other examples of art-recognized antagonists to other biologically active receptors include propranolol for the  $\beta$ -adrenergic receptor, cimetidine for the Histamine-H<sub>2</sub> receptor and the like.

The term "Angiotensin II" refers to the biologically active ligand which is an octapeptide represented by the amino acid sequence of: Asp-Arg-Val-Tyr-Ile-His-Pro-Phe. Similarly, the term "Angiotensin III" refers to the biologically active

-13-

ligand which is a heptapeptide represented by the amino acid sequence of: Arg-Val-Tyr-Ile-His-Pro-Phe.

It is to be understood that the disclosure of the present invention employs the conventional abbreviations for the various common amino acids as generally accepted in the peptide art and as recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, Biochemistry II, 1726 (1972). Many other terms used throughout the present disclosure are believed to be readily understood in the art but a definition of some of these terms is provided in Table 1 for convenience sake.

TABLE 1

DEFINITION OF VARIOUS TERMS		
	<u>Term</u>	<u>Definition</u>
15	Aib	2-aminoisobutyryl
	Alk	C <sub>1</sub> -C <sub>4</sub> alkyl or alkenyl group;
	Bzl	benzyl group
	Cha	L-cyclohexylalanyl
20	DCC	N,N'-Dicyclohexylcarbodiimide
	DMF	dimethylformamide
	Et	ethyl group
	Halide	F, Br, Cl, or I;
	HBT	1-hydroxybenzotriazole
25	HPLC	high-pressure liquid chromatography
	Me	methyl group
	Mtr	4-methoxy-2,3,6-Trimethylbenzenesulfonyl;
	Pmc	2,2,5,7,8-pentamethylchroman-6-sulfonyl;
	Pr	propyl
30	t-Boc	t-Butoxycarbonyl
	TFA	trifluoroacetic acid
	Tos	tosyl group
	Trt	trityl group

-14-

As noted previously, the chemical interaction between the ligand and the receptor can cause the desensitization of the receptor such that a subsequent interaction of the ligand on the receptor results in attenuation of the subsequent biological response to the ligand. One such biologically active ligand which serves to illustrate the effects of desensitization of a receptor is GnRH.

As mentioned earlier, GnRH is released from the hypothalamus and binds to a receptor on the pituitary gland, causing the release of LH (Luteinizing Hormone) and FSH (Follicle-Stimulating Hormone). GnRH has been purified and identified in mammals to be a decapeptide having the amino acid sequence: Pyr-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH<sub>2</sub>. The following are other naturally occurring analogs of GnRH:

Salmon GnRH: Pyr-His-Trp-Ser-Tyr-Gly-Trp-Leu-Pro-Gly-NH<sub>2</sub>;

Chicken GnRH-II: Pyr-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH<sub>2</sub>; and

Chicken GnRH-I: Pyr-His-Trp-Ser-Tyr-Gly-Leu-Gln-Pro-Gly-NH<sub>2</sub>.

However, initial attempts at using GnRH to induce ovulation were unsuccessful because of the failure to recognize the necessity of pulsatile administration. Upon recognizing the therapeutic potential of GnRH, biochemists began synthesizing both agonistic and antagonistic analogs of GnRH.

One approach to this goal has been the replacement of an amino acid residue of GnRH with another amino acid. Although in a few instances decapeptides containing such a replacement have been found to be more active than GnRH, due primarily to an



-15-

increase in resistance to degradation by enzymes in vitro and in vivo, e.g., [D-Ala<sup>6</sup>]-GnRH, A. Arimura, et al., Endocrinology, 95, 1174 (1971) and [D-Leu<sup>6</sup>]-GnRH, J. A. Vilchez-Martinez et al., Biochem. Biophys. Res. Commun, 59, 1226 (1974), for the most part the replacement containing decapeptides have been less active.

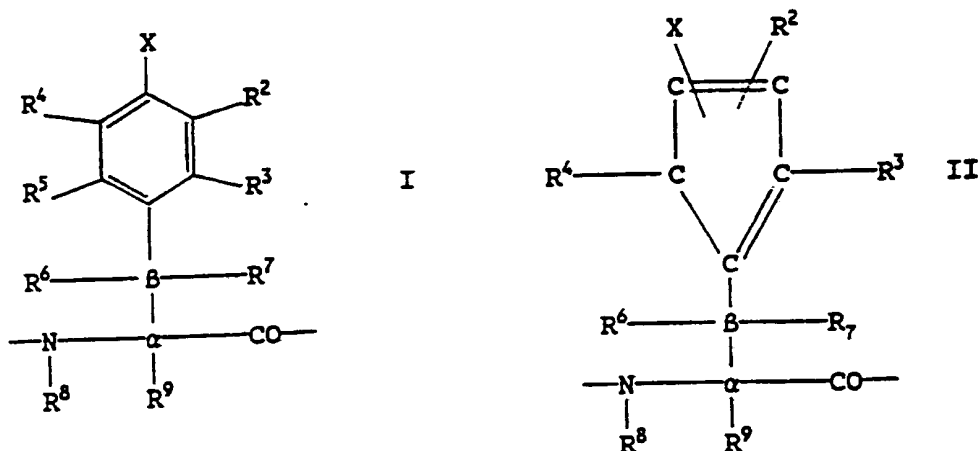
Thus, GnRH analogs synthesized previously have been designed on the basis of resistance to metabolic degradation, and have been particularly concerned with the incorporation of a D-amino acid into the GnRH peptide (as mentioned above), or with N-methylation of one or more of the peptide bonds in order to invoke intestinal stability to enzymes (European Patent No. 0 328 090). The criteria for selection of the analogs described herein is distinctly different from previous work such as that of EP-0,328,090 in that selection is based on GnRH analogs with receptor desensitizing properties, achieved principally by modifying the Tyr<sup>5</sup> or His<sup>5</sup> residue of GnRH peptides in the manner according to Formulas I and II.

Contrary to the initial intent of the previous workers, even when more active agonists of GnRH were synthesized using the criteria for developing agonists and antagonists with increased resistance to degradation by enzymes, many were found to have disadvantageous long-term effects. More specifically, these analogs of GnRH stimulated the release of LH and FSH initially, but continuous administration resulted in desensitization of GnRH receptors on gonadotropes and decreased gonadotropin secretion. Accordingly, prolonged use of high concentrations of GnRH agonists has caused reversible ovarian and uterine atrophy in

-16-

animals and has prevented implantation of ova and gestation. Likewise, high concentrations of previously synthesized GnRH agonists in males has inhibited testicular synthesis of testosterone.

5 However, through extensive research, the present inventors have discovered a method for selecting non-desensitizing analogs of a biologically active ligand containing at least one 6-membered aromatic ring or 5-membered ring. The method comprises  
 10 selecting an analog of a biologically active ligand containing a 6-membered aromatic ring according to Formula I or selecting an analog of a biologically active ligand containing a 5-membered ring according to Formula II:



15 In Formulas I and II, above, X is selected from H, R<sup>1</sup>, -OR<sup>1</sup>, halide, -CN, -CHO, C(halide)<sub>3</sub>, -alk-OH, -alk-OR<sup>1</sup>, -alk-CO<sub>2</sub>H, -alk-CO<sub>2</sub>R<sup>1</sup>, -alk-SH, -alk-SR<sup>1</sup>, -alk-CONH<sub>2</sub>, -CO<sub>2</sub>H, -CO<sub>2</sub>R<sup>1</sup>, -COR<sup>1</sup>, -OCONH<sub>2</sub>, -OCH<sub>2</sub>OH, -OCH<sub>2</sub>OR<sup>1</sup>, -OCOR<sup>1</sup>, -N<sub>3</sub>, -N<sub>2</sub>, -NHCOR<sup>1</sup>, -NO<sub>2</sub>, -NH<sub>2</sub>, -NHR<sup>1</sup>,  
 20 -NR<sup>1</sup><sub>2</sub>, -SO<sub>3</sub>H, -SO<sub>2</sub>R<sup>1</sup>, -SCOR<sup>1</sup>, -NCS, -SCSR<sup>1</sup>, -SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NHR<sup>1</sup>, -SO<sub>2</sub>NR<sup>1</sup><sub>2</sub>, -SO<sub>4</sub>H, -PO<sub>3</sub>H, -PO<sub>4</sub>H<sub>2</sub>, -SH, -SR<sup>1</sup>, -N=N-R<sup>1</sup>, =O, =NH, =N-R<sup>1</sup>, =N-OH, =N-OR<sup>1</sup>, -CONH<sub>2</sub>, -CONHR<sup>1</sup>, -CONR<sup>1</sup><sub>2</sub>,

-17-

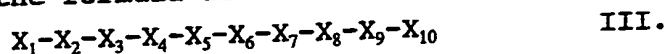
-NHSO<sub>2</sub>R<sup>1</sup>, -SiR<sup>1</sup><sub>3</sub>, tetrazole, imidazole, or substituted or unsubstituted phenyl, -alkylphenyl, -O-phenyl, -O-alkylphenyl. Additionally, R<sup>1</sup> is selected from alkyl of 1-7 carbon atoms, alkenyl or alkynyl of 2-7 carbon atoms or cycloalkyl of 3-7 carbon atoms, optionally halogenated at one or more hydrogen. Likewise, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup> and R<sup>9</sup> are groups selected from X or -OH. In the above,  $\alpha$  is selected from P, N, S, or C being in either the L- or D-configuration and  $\beta$  can be deleted or extended by 1-2 carbon atoms, substituted or unsubstituted. Moreover, the nitrogen atom attached to  $\alpha$  can be substituted or unsubstituted C, S, O or P and the carbon atom attached to  $\alpha$  and oxygen can be substituted or unsubstituted N, S, O, P or C. The aromatic ring of Formula I or the ring of Formula II can contain 0-4 N, S or O atoms. Likewise, the aromatic ring of Formula I or ring of Formula II can be fused with a 6-membered aromatic ring which can contain 0-4 N, S or O atoms and which can be substituted in the same manner as the aromatic ring of Formula I. Also included within Formulas I and II are D-tyrosine and D-Histidine.

The analogs of a biologically active ligand are selected so as to contain a ring modified according to Formulas I or II and so that the resulting biologically active ligand has reduced desensitizing properties, i.e., the biologically active ligand is such that the subsequent interaction of the ligand with the receptor does not substantially attenuate the biological response. The discovery and use of analogs according to Formulas I or II, and especially GnRH analogs according to Formulas I or II, with reduced desensitizing properties is new.

-18-

The analog of a biologically active ligand containing at least one 6-membered aromatic ring can be selected, for example, from the peptides Angiotensin (II or III) or GnRH. Thus, one method according to the present invention is the selection of a non-desensitizing analog of the biologically active ligand GnRH wherein the tyrosine residue at the 5-position has been modified according to Formula I.

More specifically, some preferred analogs selected by the present method include GnRH analogs represented by the formula III:



In Formula III, above,  $X_1$ - $X_{10}$  (positions 1-10) are selected from the following:  $X_1$  is Pyr or N-acyl-amino acid;  $X_2$  is an aromatic amino acid;  $X_3$  is an aromatic amino acid,  $X_4$  is Ala, Thr, Ser, Hse, Ser(Me),  $\alpha$ -C-methyl-Ser(Me), Aib;  $X_5$  is Tyr(O-alkyl), Phe(4'-halogen); Phe(4'-alkyl);  $X_6$  is Gly, Aib, or D-amino acid (natural or synthetic, including an amino acid bearing a protecting group),  $\alpha$ -C-alkyl-amino acid;  $X_7$  is Leu, Ile, Trp, Cha,  $\alpha$ -C-methyl-amino acid, Val;  $X_8$  is Arg, Gln, Tyr, Leu;  $X_9$  is Pro, pipecolic acid, nipecotic acid, azetidine-2-carboxylic acid, dehydroproline, thioproline, hydroxyproline; and  $X_{10}$  is N(H)R wherein R is GlyNH<sub>2</sub>, azaGlyNH<sub>2</sub>, alkyl, haloalkyl, hydroxyalkyl, cycloalkyl or aryl, wherein each alkyl or aryl group has from 1 to 7 carbon atoms.

Another particularly preferred embodiment is one in which the  $\alpha$ -carbon atom of at least one of the amino acids is alkylated, in which case position 5 may be as described by Formulas I or II or may be  $\alpha$ -C-methyl-histidine or  $\alpha$ -C-methyl-tyrosine, or the like. The modifications illustrated for the tyrosine

-19-

residue of GnRH are also applicable to other Tyr-containing peptides.

In a similar fashion, the role of Tyr in receptor desensitization can also be carried out by Histidine in certain peptides, of which chicken GnRH-II is an example. Therefore, modifications to the imidazole group which remove the hydrogen bond donor capability of imidazole, for example alkylation at N<sub>1</sub>, or N<sub>3</sub>, can be expected to diminish the desensitizing effects of certain ligands containing imidazole.

More generally, selective modification or substitution of the hydroxyl groups in a biologically active ligand containing a 6-membered aromatic ring or 5 membered ring according to Formulas I and II, respectively, may produce analogs with reduced desensitizing effects. Such low-desensitizing analogs may also be used to attenuate the desensitization evoked by a high-desensitizing ligand, as illustrated for GnRH in Examples 4 and 5, *infra*.

Additionally, selection of a biologically active ligand having a phenolate group according to Formula I or a 5-membered ring according to Formula II may likewise favorably alter the duration of action of the ligand. Thus, in the case of an agonistic ligand, the duration of the biological response is increased. For example, [Tyr(Me)<sup>5</sup>]-GnRH has a longer duration of action than GnRH. This increase in duration of action and the non-desensitizing nature of the analog [Tyr(Me)<sup>5</sup>]-GnRH is illustrated in Example 2 and, *infra*.

The same method is also applicable to antagonist analogs of a ligand. In the case of an antagonist ligand, however, the duration of action is

-20-

decreased. An example is seen with angiotensin, wherein methylation of the tyrosine OH of the angiotensin antagonist [Sar<sup>1</sup> Ile<sup>8</sup>] ANG II to give [Sar<sup>1</sup> Tyr(Me)<sup>4</sup> Ile<sup>8</sup>] ANG II results in a reduction of the desensitization properties and duration of action. The decrease in the duration of action and the non-desensitizing nature of the above antagonistic analogs is illustrated in Example 1, infra.

It is important to understand, however, that the present invention is not limited to peptides such as GnRH and Angiotensin II or III, but may be applied to any desensitizing biologically active ligands having a 6-membered aromatic ring or 5-membered ring. Accordingly, other examples demonstrating the extensive applicability of the selection method according to the present invention are as follows.

Additional examples of non-desensitizing analogs of a biologically active ligand selected according to the present invention include, but are not limited to, the opioid ligands, wherein desensitization, tolerance, dependence, and withdrawal may be effectively reduced by modification of the phenolic group or conjugated hydroxyl group of an opiate peptide (which have the N-terminal sequence Tyr-Gly-Gly-Phe-Met/Leu such as enkephalins, dynorphins and endorphins) or non-peptide ligands. Thus, methylation of a phenolic hydroxyl group of morphine produces the less active analog codeine. Furthermore, deletion of both hydroxyl groups in morphine can result in the agonistic mimetic methadone which displays increased potency and decreased withdrawal symptoms due to the blockade of morphine dependence. Accordingly, it is understood in the present disclosure that GnRH is used as an example only, and that the structural

-21-

modifications to the 6-member aromatic ring in GnRH can likewise be applied to any biologically active ligand having a 6-membered aromatic ring or 5-membered ring which can be modified according to Formulas I or II.

5           As was mentioned, such biologically active ligands are likewise not limited to merely the peptidic ligands but also include non-peptidic ligands. Consequently, the present invention is useful in selecting agonistic non-peptidic analogs of a  
10 biologically active ligand containing at least one 6-membered aromatic ring according to Formula I or 5-membered ring according to Formula II in which the interrelated properties of desensitization, tolerance, dependence, withdrawal and addiction are reduced.

15           As used herein, the term "non-peptidic ligands" as applied to the Formulas I and II refer to the ring structures depicted in Formulas I and II wherein the peptide backbone has been removed. For example, representative examples of non-desensitizing  
20 analogs of a biologically active ligand selected according to the present invention include, but are not limited to, certain steroid hormones and catecholamine hormones. Such modifications where applicable, may also be applied to a biologically active ligand  
25 containing a conjugated hydroxyl group, of which -C=C-C-OH is an example.

To further illustrate the far-reaching effects of the present invention, GnRH will once again be used as a representative example to display the  
30 numerous advantages which can be achieved by the practice of the present invention. GnRH analogs are useful for increasing sexual activity, fertility and egg yield and inducing ovulation in animals, and are

-22-

useful for increasing productivity in the farming of fish, poultry and mammals, as well as for increasing fertility in humans. GnRH analogs can also increase lifetime gamete production, and permit collection of gametes from pre- and post-natal, juvenile, prepubertal and mature mammals for natural and artificial fertilization. GnRH analogs are also effective in the treatment of gonadotropin-related diseases such as prostatic cancer, breast cancer, endometriosis and fibroid shrinkage.

However, as was mentioned, agonistic and super agonistic analogs of GnRH often require continuous or rapid pulsatile dosing in order to avoid the potent desensitizing effects associated with these peptides. The development of GnRH agonist analogs with reduced receptor desensitizing properties is advantageous because such analogs obviate the need for continuous or rapid pulsatile dosing methods and may be administered in a single dose or with considerably lower dose frequency. The advantage of eliminating the need for pulsatile dosing likewise extends to all analogs of a biologically active ligand according to the present invention.

The use of the present method of selecting a non-desensitizing analog of a biologically active ligand quite surprisingly possesses the additional advantage of not requiring the continued use of solely the non-desensitizing analog but allows for the use of "normally" desensitizing analogs without the expected desensitizing effects upon the biologically active receptor. For example, a non-desensitizing GnRH analog may be administered alone or in combination with another normally desensitizing GnRH agonist since the desensitizing properties of the latter are suppressed



-23-

by the presence of, or pretreatment with, the former. Stated in another way, by treating a patient with a non-desensitizing GnRH analog according to the present invention, it is possible to subsequently or

5 simultaneously treat the patient with an normally desensitizing analog of GnRH because the pretreatment with, or presence of, the non-desensitizing GnRH analog according to the present invention acts to eliminate or suppress the desensitizing effect of the normally

10 desensitizing GnRH analog. A "normally" desensitizing analog is an analog of GnRH which has not been modified in a manner which reduces the desensitizing properties as illustrated in the present invention. The use of a non-desensitizing GnRH analog in combination with

15 normally desensitizing GnRH or synthetic analogs thereof is a preferred method aspect of the present invention.

A "biologically active receptor" according to the present invention is a molecule, having a specific

20 binding site for its complementary ligand, and includes classical hormonal receptors, binding and/or transport proteins, enzymes, antibodies and the like. One embodiment of a biologically active receptor includes membrane-bound proteins which control certain cellular

25 processes in which themselves are regulated by the binding, or lack of binding of a complementary naturally occurring biologically active ligand. Because such membrane bound biologically active receptors are bound to membrane, it is believed that

30 the conformation of the biologically active ligand necessary to activate such receptors are lipid induced. On the other hand, there are other biologically active receptors which are not membrane bound. In such cases, such receptors may not require a lipid induced

35 conformation of the biologically active ligand and, in

-24-

fact, may require an aqueous induced conformation of the complementary biologically active ligand in order to activate such receptors.

Examples of biologically active receptors have been well documented in the art. Specific examples include insulin receptor (wherein the complementary ligand is insulin) and the Angiotensin II receptor (wherein the complementary ligand is Angiotensin II), and the like.

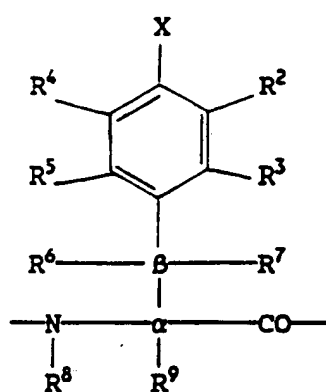
As described above, the non-desensitizing analogs of a biologically active ligand selected according to the present invention have a multitude of uses in humans and animals, either of which are considered "patients" as that term is employed in the present disclosure or claims. Additional examples of desired uses of non-desensitizing GnRH analogs of a biologically active ligand selected according to the present invention include, for example, the ability to more readily synchronize estrus in livestock, e.g., cattle, sheep or swine, either in order to be able to mate all the females in a given group with a male of the desired genetic quality, or so as to be able to perform artificial insemination on a maximum number of females, both within a comparatively short period of time. It will be appreciated that such a method is of particular importance for breeders of race horses and show animals, where the fee is paid for the services of an exceptional male animal often amount to considerable sums of money.

Thus, in accordance with the multitude of potential uses for non-desensitizing analogs of biologically active ligands, the present invention is also directed to a method of treating a patient in a

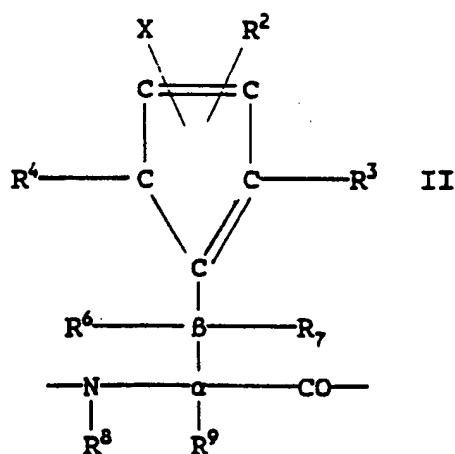
-25-

non-desensitizing fashion with a non-desensitizing analog of a biologically active ligand containing at least one 6-membered aromatic ring or 5-membered ring.

- The method comprises first selecting an analog of a  
 5 biologically active ligand containing a 6-membered aromatic ring according to Formula I or selecting an analog of a biologically active ligand containing a 5-membered ring according to Formula II:



I



II

- In Formulas I and II, above,  $X$  is selected  
 10 from  $H$ ,  $R^1$ ,  $-OR^1$ , halide,  $-CN$ ,  $-CHO$ ,  $C(\text{halide})_3$ ,  $-alk-OH$ ,  $-alk-OR^1$ ,  $-alk-CO_2H$ ,  $-alk-CO_2R^1$ ,  $-alk-SH$ ,  $-alk-SR^1$ ,  $-alk-CONH_2$ ,  $-CO_2H$ ,  $-CO_2R^1$ ,  $-COR^1$ ,  $-OCONH_2$ ,  $-OCH_2OH$ ,  $-OCH_2OR^1$ ,  $-OCOR^1$ ,  $-N_3$ ,  $-N_2$ ,  $-NHCOR^1$ ,  $-NO_2$ ,  $-NH_2$ ,  $-NHR^1$ ,  $-NR^1_2$ ,  $-SO_3H$ ,  $-SO_2R^1$ ,  $-SCOR^1$ ,  $-NCS$ ,  $-SCSR^1$ ,  $-SO_2NH_2$ ,  
 15  $-SO_2NHR^1$ ,  $-SO_2NR^1_2$ ,  $-SO_3H$ ,  $-PO_3H$ ,  $-PO_4H_2$ ,  $-SH$ ,  $-SR^1$ ,  $-N=N-R^1$ ,  $=O$ ,  $=NH$ ,  $=N-R^1$ ,  $=N-OH$ ,  $=N-OR^1$ ,  $-CONH_2$ ,  $-CONHR^1$ ,  $-CONR^1_2$ ,  $-NHSO_2R^1$ ,  $-SiR^1_3$ , tetrazole, imidazole, or substituted or unsubstituted phenyl,  $-alkylphenyl$ ,  $-O-phenyl$ ,  $-O-alkylphenyl$ ,  $-O-benzyloxy-carbonyl$ . Additionally,  $R^1$   
 20 is selected from alkyl of 1-7 carbon atoms, alkenyl or alkynyl of 2-7 carbon atoms or cycloalkyl of 3-7 carbon atoms, optionally halogenated at one or more hydrogen. Likewise,  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^8$  and  $R^9$  are groups

-26-

selected from X or -OH. In the above,  $\alpha$  is selected from P, N, S, or C being in either the L- or D-configuration and  $\beta$  can be deleted or extended by 1-2 carbon atoms, substituted or unsubstituted. Moreover, the nitrogen atom attached to  $\alpha$  can be substituted or unsubstituted C, S, O or P and the carbon atom attached to  $\alpha$  and oxygen can be substituted or unsubstituted N, S, O, P or C. The aromatic ring of Formula I or the ring of Formula II can contain 0-4 N, S or O atoms. Likewise, the aromatic ring of Formula I or ring of Formula II can be fused with a 6-membered aromatic ring which can also contain 0-4 N, S or O atoms which can be substituted in the same manner as the aromatic ring of Formula I. Also included within Formulas I and II, above, are D-tyrosine and D-histidine.

Step two of the method comprises administering to a patient in need thereof the selected analog of a biologically active ligand as a composition comprising (i) a pharmaceutically effective amount of the selected analog and (ii) a pharmaceutically acceptable carrier for the selected analog. While it is possible for the particular active ingredient to be administered as the raw chemical, it is preferable, in view of the potency thereof, to present it as pharmaceutical formulation containing an acceptable carrier.

In a preferred method, at least one non-desensitizing analog of GnRH is given in combination with at least one normally desensitizing GnRH or synthetic analog thereof. The non-desensitizing analog(s) of GnRH may be given before a normally desensitizing GnRH analog, simultaneously therewith, or both.

-27-

For each of the utilities mentioned above, the amount of the analog of the biologically active ligand will, of course, vary both with the particular active ingredient and with the route of administration.

5 In general, however, for each of these utilities the dosage for nasal or parental administration will be in the range of about 0.005 to 200 $\mu$ g per kilogram body weight of the patient, preferably between about 0.01 and 100  $\mu$ g/kg. For oral or vaginal administration the

10 dosage would generally be in the range of about 0.005 to 1000 $\mu$ g/kg, preferably about 0.05 to 200 $\mu$ g/kg. It is understood that all dosages are calculated with reference to the base peptide. These doses apply to both a non-desensitizing and normally desensitizing

15 analog of GnRH when administered in one of the combination protocols described above.

It should be recognized that by "non-desensitizing fashion", it is understood that the present method treats a patient in a manner such that

20 the patient does not need to be treated with the type of pulsatile dosing that was heretofore required in order to successfully achieve the hormonal changes in the body of animals and humans. Rather, the analogs, alone or in combination with normally desensitizing

25 analogs, according to the present invention obviate the need for continuous or rapid pulsatile dosing methods and thus may be administered in a single dose or with considerably lower dose frequency.

The formulations of the present invention

30 comprise an active ingredient or combination thereof, as described above, together with one or more pharmaceutically acceptable carriers as well as other optional therapeutic ingredients. The carriers must be pharmaceutically "acceptable" in the sense of being

-28-

compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. Desirably, the formulations should not include oxidizing agents and other substances with which peptides, or non-peptides, are known to be incompatible.

The formulations include those suitable for oral, rectal, nasal, topical, vaginal or parenteral (including subcutaneous, intramuscular, and intravenous) administration, although the more suitable route in any given case will depend upon the selected active ingredient.

As another preferred route of administration, an active ingredient(s) may be presented as a depot formulation having a slow-release characteristic suiting it for implantation in the body of the recipient, for example, sub-cutaneously, intramuscularly, intraperitoneally or intravaginally. For example, injections may be given in depot form with a slow release agent such as an emulsion, sesame oil, a 10% aqueous polyvinylpyrrolidone, or in biodegradable microspheres such as polylactic/glycolic acid. Alternatively, administration may be given with the aid of a mini-osmopump, located internally or externally. In animals such as fish, administration may be optionally given with a dopamine antagonist such as domperidone.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Typical methods include the step of bringing into association the active ingredient with the carrier which optionally contains one or more accessory

-29-

ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired formulation.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; or as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-water liquid emulsion or a water-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compressing or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert dilution, lubricating, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powder compound moistened with an inert liquid diluent.

Formulations for rectal administration may be presented as a suppository with the usual carriers such as coco-butter, while a suitable formulation for nasal administration is nasal drops comprising an active ingredient in an aqueous or oily solution.

Formulations suitable for oral administration include lozenges comprising the active ingredient in a flavored basis, usually sucrose or acacia or

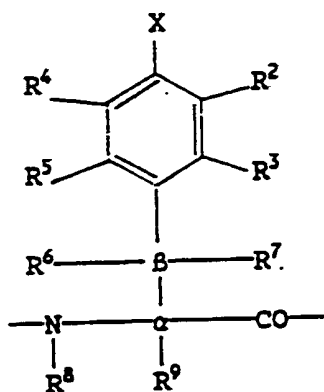
-30-

tragacanth; and pastilles comprising the active ingredient in an inert basis such as gelatin, glycerine or sucrose and acacia.

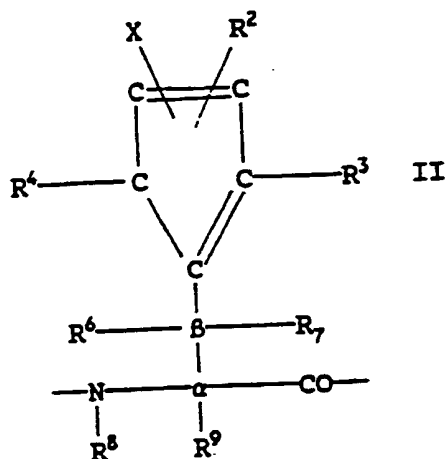
- Formulations suitable for vaginal administration may be presented as pessaries, creams, pastes, or spray formulations containing in addition to the active ingredients such carriers as are known in the art to be appropriate.

- Formulations suitable for parental administration conveniently comprise sterile aqueous solutions of the active ingredient, which solutions are preferably isotonic with the blood of the recipient. Such formulations may be conveniently prepared by dissolving a solid active ingredient in water to produce an aqueous solution, and rendering said solution sterile and isotonic with the blood of the recipient.

- Also provided herein are new, non-desensitizing analogs of biologically active ligands containing at least one 6-membered aromatic ring or 5-membered ring. The non-desensitizing analogs of a biologically active ligand contain at least one 6-membered aromatic ring according to Formula I or 5-membered ring according to Formula II:



I



II



-31-

In Formulas I and II, above, X is selected from H, R<sup>1</sup>, -OR<sup>1</sup>, halide, -CN, -CHO, C(halide)<sub>3</sub>, -alk-OH, -alk-OR<sup>1</sup>, -alk-CO<sub>2</sub>H, -alk-CO<sub>2</sub>R<sup>1</sup>, -alk-SH, -alk-SR<sup>1</sup>, -alk-CONH<sub>2</sub>, -CO<sub>2</sub>H, -CO<sub>2</sub>R<sup>1</sup>, -COR<sup>1</sup>, -OCONH<sub>2</sub>, -OCH<sub>2</sub>OH, -OCH<sub>2</sub>OR<sup>1</sup>, -OCOR<sup>1</sup>, -N<sub>3</sub>, -N<sub>2</sub>, -NHCOR<sup>1</sup>, -NO<sub>2</sub>, -NH<sub>2</sub>, -NHR<sup>1</sup>, -NR<sup>1</sup><sub>2</sub>, -SO<sub>3</sub>H, -SO<sub>2</sub>R<sup>1</sup>, -SCOR<sup>1</sup>, -NCS, -SCSR<sup>1</sup>, -SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NHR<sup>1</sup>, -SO<sub>2</sub>NR<sup>1</sup><sub>2</sub>, -SO<sub>4</sub>H, -PO<sub>3</sub>H, -PO<sub>4</sub>H<sub>2</sub>, -SH, -SR<sup>1</sup>, -N=N-R<sup>1</sup>, =O, =NH, =N-R<sup>1</sup>, =N-OH, =N-OR<sup>1</sup>, -CONH<sub>2</sub>, -CONHR<sup>1</sup>, -CONR<sup>1</sup><sub>2</sub>, -NHSO<sub>2</sub>R<sup>1</sup>, -SiR<sup>1</sup><sub>3</sub>, tetrazole, imidazole, or substituted or unsubstituted phenyl, -alkylphenyl, -O-phenyl, -O-alkylphenyl, -O-benzyloxy-carbonyl. Additionally, R<sup>1</sup> is selected from alkyl of 1-7 carbon atoms, alkenyl or alkynyl of 2-7 carbon atoms or cycloalkyl of 3-7 carbon atoms, optionally halogenated at one or more hydrogen.

Likewise, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup> and R<sup>9</sup> are groups selected from X or -OH. In the above, α is selected from P, N, S, or C being in either the L- or D-configuration and β can be deleted or extended by 1-2 carbon atoms, substituted or unsubstituted. Moreover, the nitrogen atom attached to α can be substituted or unsubstituted C, S, O or P and the carbon atom attached to α and oxygen can be substituted or unsubstituted N, S, O, P or C. The aromatic ring of Formula I or the ring of Formula II can contain 0-4 N, S or O atoms.

Likewise, the aromatic ring of Formula I or ring of Formula II can be fused with a 6-membered aromatic ring which can also contain 0-4 N, S or O atoms which can be substituted in the same manner as the aromatic ring of Formula I. Also included within the Formulas I and II, above, are D-tyrosine and D-histidine.

Excluded from the compounds according to this aspect of the present invention are those antagonist analogs of mammalian GnRH obtained by substituting

-32-

D-amino acids at positions 1, 2 or 3, and analogs which have position 2 deleted. Also excluded are the following agonist analogs based on mammalian GnRH:

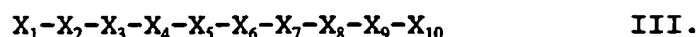
- (A) [D-Phe<sup>6</sup>]GnRH in which the hydroxyl group of tyrosine is protected with benzyl, acetyl, tosyl, benzoyl, t-butyl, tetrahydropyran-2-yl, trityl, 2,4-dichlorobenzyl, benzyloxycarbonyl or 2-bromobenzyloxycarbonyl;
- (B) [Phe<sup>5</sup>]GnRH, [Ala<sup>4</sup>Phe<sup>5</sup>]GnRH and [Phe(N<sub>3</sub>)<sup>5</sup>]GnRH;
- (C) [Des<sup>10</sup>Phe<sup>5</sup>-D-Ala<sup>6</sup>ProNHR<sup>9</sup>]GnRH in which position 2 is His, Tyr, Trp or Phe(4'-NH<sub>2</sub>), position 7 is Leu, Ile or Nle, and R is Et, Pr, CH<sub>2</sub>CH<sub>2</sub>OH or CHMe<sub>2</sub>;
- (D) [Des<sup>10</sup>X<sup>5</sup>Pro-B<sup>9</sup>]GnRH in which position 3 is Trp, Phe or 3(1-naphthylalanine); X is His, Phe or Phe(F<sub>5</sub>); position 6 is D-Ala(Y) wherein Y is naphthyl, anthryl, fluorenyl, phenanthryl, biphenyl, benzhydryl, phenyl or cyclohexyl with 3 or more alkyl groups, perhydronaphthyl, adamantyl, perhydrobenzhydryl, phenyl, cyclohexyl, dicyclohexyl, heterocycle aryl, optionally N-alkylated alkylamine or cycloalkylamine, or NHCH[(CH<sub>2</sub>)<sub>n</sub>NHC(=NR<sub>2</sub>)R<sub>1</sub>]CO wherein: n is 1-5; R<sub>1</sub> is alkyl or NRR<sub>3</sub> wherein R is H or alkyl and R<sub>3</sub> is H, alkyl, fluoroalkyl, cycloalkyl, phenyl, benzyl, alkyl-morpholino or (CH<sub>2</sub>)<sub>n</sub>(R<sub>4</sub>)<sub>2</sub> wherein n is 1-5 and R<sub>4</sub> is alkyl; R<sub>2</sub> is H or R<sub>3</sub>; or wherein C=NR<sub>2</sub>R<sub>1</sub> can be a ring; position 7 is Leu, Ile, MeLeu or Trp; position 8 is Arg, Gln, Tyr or Leu; and B is selected from GlyNH<sub>2</sub>, NHB<sub>2</sub> wherein B<sub>2</sub> is alkyl, cycloalkyl or haloalkyl, and NHCONHB<sub>3</sub> wherein B<sub>3</sub> is H or alkyl;
- (E) analogs containing a γ-lactam in the 6-7 position, in which position 1 is Pyr, N-acetyl, N-Pyr-imino acid, or (C<sub>3-7</sub> cycloalkyl)acyl; positions 2 and 3 are aliphatic or aromatic amino acid; position 4 is Ser, Thr or Ala; position 5 is aromatic amino acid His, Trp or Phe; position 8 is an amino acid with a

-33-

basic sidechain; position 9 is imino acid or aliphatic amino acid; position 10 is GlyNH<sub>2</sub>, AlaNH<sub>2</sub>, NH<sub>2</sub>Et, NHPr, NHCH<sub>2</sub>CH<sub>2</sub>OH; and

- (F) analogs in which position 5 is Phe, Tyr(Me), N-alkyl-Phe, N-alkyl-Tyr(Phe) or N-alkyl-Tyr(Et), wherein the nitrogen atom of at least one of the amide bonds is alkylated.

Some preferred novel analogs selected by the present invention include GnRH analogs having the Formula III:



In Formula III, above, X<sub>1</sub>-X<sub>10</sub> (positions 1-10) are selected from the following: X<sub>1</sub> is Pyr or N-acyl-amino acid; X<sub>2</sub> is an aromatic amino acid; X<sub>3</sub> is an aromatic amino acid, X<sub>4</sub> is Ala, Thr, Ser, Hse, Ser(Me), α-C-methyl-Ser(Me) or Aib; X<sub>5</sub> is Tyr(O-alkyl), Phe(4'-halogen) or Phe(4'-alkyl); X<sub>6</sub> is Gly, Aib, or D-amino acid (natural or synthetic, including an amino acid bearing a protecting group) or α-C-alkyl-amino acid; X<sub>7</sub> is Leu, Ile, Trp, Cha, Val or α-C-methyl-amino acid; X<sub>8</sub> is Arg, Gln, Tyr or Leu; X<sub>9</sub> is Pro, pipecolic acid, nipecotic acid, azetidine-2-carboxylic acid, dehydroproline, thioproline or hydroxyproline; and X<sub>10</sub> is N(H)R wherein R is GlyNH<sub>2</sub>, azaGlyNH<sub>2</sub>, alkyl, haloalkyl, hydroxyalkyl, cycloalkyl or aryl, wherein each alkyl or aryl group has from 1 to 7 carbon atoms.

Another preferred embodiment is one in which the α-carbon atom of at least one of the amino acids is alkylated, in which case position 5 may be as described by Formulas I or II or may be α-C-methyl-histidine or α-C-methyl-tyrosine. GnRH analogs and other analogs of biologically active ligands containing α-C-alkyl-amino acids are new. A particularly preferred embodiment

-34-

includes GnRH analogs in which at least one of positions 4, 5, 6 and 7 contain an  $\alpha$ -C-alkyl-amino acid. Such modifications are advantageous for promoting and stabilizing turns occurring in this region of the peptide backbone, and for limiting proteolysis of the peptide.

Many of the analogs of a biologically active ligand according to the present invention can be synthesized by the methods well-known to one skilled in the art. For example, compounds can be synthesized by the solid phase method by one or more strategies known to one skilled in the art (J. Stewart and J. Young, Solid Phase Peptide Synthesis, 2nd Ed. (1984), Pierce Chemical Co.). In one such example, chloromethylated polystyrene or benzhydrylamine resin can be used with N-t-butyloxycarbonyl protected amino acids and sidechain protection such as His(Tos), Ser(Bzl) and Arg(Tos). The peptide-resin bond may be cleaved and protecting groups removed by treatment with anhydrous HF, or the peptide-resin bond may be cleaved and aminoalkylated with alkylamine and the protecting groups subsequently removed with HF. The peptide may then be purified by reversed-phase HPLC.

Purification of peptides can be accomplished, for example, with the use of a Varian HPLC system equipped with a Vista 401 micro-processor controller. Separations can be achieved, for example, on a Bio-Rad Hi-Pore 318 reverse-phase preparative column (25.0 x 2.15 cm) at 25°C with a stepped linear gradient of acetonitrile in 0.1%  $\text{CF}_3\text{CO}_2\text{H}$  at a flow rate of 7.5 ml/min. Automated repetitive injections of peptides (5 x 5 mg) can then be made from a nitrogen pressurized Rheodyne injector with a 2.0 ml sample loop. One-fifth

-35-

of the total sample may then be injected during each run by lowering the flow rate to 4.0 ml/min for a 0.1-min "inject" period. One cycle could thus consist of the following events: 0 - 10 min, 7.5 ml/min, 90% H<sub>2</sub>O/10% of 1% aqueous CF<sub>3</sub>CO<sub>2</sub>H; 10 - 11 min, 4.0 ml/min; 11 - 11.1 min, "inject"; 11.1 - 13 min, 7.5 ml/min, 70% H<sub>2</sub>O/20% CH<sub>3</sub>CN/10% of 1% CF<sub>3</sub>CO<sub>2</sub>H; 13 - 30 min, 45% H<sub>2</sub>O/45% CH<sub>3</sub>CN/10% of 1% CF<sub>3</sub>CO<sub>2</sub>H; 30 - 42 min, 90% CH<sub>3</sub>CN/10% of 1% CF<sub>3</sub>CO<sub>2</sub>H; 42-50 min, 100% H<sub>2</sub>O.

10

In a novel solid phase peptide synthesis method according to the present invention, synthesis is carried out at room temperature, the resin is a novel chloro-(ortho-chloro)-trityl-resin to which the FMOC-protected, terminal amino acid is attached in the presence of diethylpropylamine (1.1 equivalents) in methylene chloride for 1 hour.

Traditionally, two solid phase peptide synthesis strategies using Boc-amino acids and FMOC-amino acids, respectively, have been used. In the Boc strategy, the peptide-resin bond is cleaved with the use of strong acid conditions, such as anhydrous HF, such that the protecting groups are simultaneously removed. In the FMOC-strategy, the peptide-resin is cleaved with the use of intermediate acid conditions, such as TFA, such that most or all of the protecting groups are simultaneously removed. Using the novel (ortho-chloro)trityl resin, the conditions for cleaving the peptide-resin bond is so mild that even the most acid-sensitive protecting groups commonly used in solid phase peptide synthesis remain attached to the peptide. Consequently, the ability to retain the acid-sensitive protecting groups has thus allowed the present inventors to synthesize new and useful analogs

-36-

biologically active ligands such as GnRH and opiate peptides.

Using the (orthochloro)trityl resin, stepwise synthesis of the peptide with FMOC-amino acids (2.5 equivalents) is carried out with DCC/HBT (2.5 equivalents) as coupling agent(s) in a suitable solvent such as DMF for 1-2 hours, and the FMOC groups removed by treatment with 20% piperidine in DMF for 10-30 minutes. Acid-sensitive sidechain protecting groups such as His(trityl), Ser(t-butyl) and Cys(t-butyl), and the like, remain intact when the peptide-resin bond is cleaved with trifluoromethanol/acetic acid/methylene chloride in a ratio of 7/1/2, respectively.

Additionally, a peptide amide may be prepared by treating the liberated peptide with methanolic HCl for 1-4 hours to yield the methyl ester followed by aminolysis in methanolic ammonia for 4-24 hours. Alternatively, treatment of the received peptide with monoalkylamine in the presence of a suitable coupling agent such as DCC/HBT in a suitable solvent such as DMF for 1-4 hours affords the peptide N-alkylamide. Removal of protecting groups may be accomplished with 50% TFA in chloroform for 30 min. When an acid-sensitive group is to be maintained, such as D-His(Trt), the synthesis is carried out with "limited" sidechain protection, for example, Pyr-His-Trp-Ser(Me)-Tyr(Me)-D-His(Trt)-Leu-Arg-Pro-resin, or alternatively by fragment condensation of Pyr-His-Trp-Ser(Me)-Tyr(Me)-D-His(Trt)-LeuOH (prepared using the (orthochloro)trityl resin) to Arg-Pro-resin or Arg-Pro-NHEt using a suitable coupling agent such as DCC/HBT and a suitable solvent such as DMF.

-37-

Synthesis of [Ser(Me)<sup>4</sup>Tyr(Me)<sup>5</sup>DHis(Trt)<sup>6</sup>-  
 ProNHET<sup>9</sup>]-GnRH is accomplished using Fmoc-amino acids  
 and (orthochochloro)trityl resin by the protocol outlined  
 above to give: Pyr-His-Trp-Ser(Me)-Tyr(Me)-D-His(Trt)-  
 5 Leu-Arg-Pro-resin which is treated with  
 F<sub>3</sub>COH/CH<sub>3</sub>CO<sub>2</sub>H/CH<sub>2</sub>Cl<sub>2</sub> (7:1:2) for 1 hour and filtered. The  
 peptide is precipitated from the filtrate with ether,  
 filtered, dissolved in DMF and treated with NH<sub>2</sub>Et and  
 DCC/HBT (2 equiv. of each) for 2 hours. The desired  
 10 product is precipitated with ether and purified by  
 reverse-phase HPLC.

The novel compounds produced according to the  
 novel process described above are analogs of GnRH, and  
 other hormonal peptides, in which an acid-sensitive  
 15 protecting group is maintained at the end of the  
 synthesis as a result of the use of an extremely labile  
 peptide-resin bond provided by the (orthochochloro)trityl-  
 resin bond. Such analogs include, but are not limited  
 to, compounds having the Formula III:

20  $X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}$  III.

In Formula III, above, X<sub>6</sub> is D-His having the  
 protecting group trityl, tosyl, dansyl, dinitrophenyl,  
 benzyloxymethyl or optionally substituted benzyl;  
 D-Ser/Thr having the protecting group trityl, t-butyl,  
 25 acyl, optionally substituted benzyl or  
 benzyloxycarbonyl; D-Cys having the protecting group  
 trityl, t-butyl, tosyl, or optionally substituted  
 benzyl; D-Tyr having the protecting group tosyl,  
 trityl, acyl, t-butyl, optionally substituted benzyl or  
 30 benzyloxycarbonyl; D-Arg having the protecting group  
 Tos, Mtr or Pmc; D-Orn/Lys having the protecting group  
 t-Boc, trityl or optionally substituted  
 benzyloxycarbonyl; D-Asp/Glu having the protecting

-38-

group t-butyl, trityl or optionally substituted benzyl;  
D-Asn/Gln having the protecting group trityl or  
xanthyl; D-Trp having the protecting group formyl or  
t-Boc;  $X_1$  is Pyr, N-acyl-amino acid, N-acyl- $\alpha$ -C-alkyl-  
5 amino acid,  $\alpha$ -C-alkyl-Pyr;  $X_2$ ,  $X_3$ ,  $X_4$ ,  $X_7$ ,  $X_8$  are  
independently natural, synthetic, protected or  
 $\alpha$ -C-alkyl- amino acids;  $X_9$  is imino acid;  $X_{10}$  is -N(H)R  
wherein R is GlyNH<sub>2</sub>, azaGlyNH<sub>2</sub>, alkyl, alkenyl,  
cycloalkyl, hydroxyalkyl, haloalkyl, aryl, wherein each  
10 alkyl or alkenyl group has from 1-7 carbon atoms; and  $X_5$   
is according to Formulas I or II, as described above,  
with the proviso that the same compounds excluded from  
Formulas I and II are likewise excluded from the  
compounds according to Formula III.

15                    Additionally, analogs of opiate peptides  
containing the acid-sensitive groups listed above  
together with modification of Tyr or His according to  
Formulas I and II are included.

                  A particularly preferred set of compounds  
20 prepared by the novel process according to the present  
invention are the compounds according to Formula III,  
wherein  $X_6$  comprises an amino acid which contains an  
acid-sensitive sidechain protecting group. The amino  
acids containing acid-sensitive protecting groups are a  
25 particularly preferred set of novel compounds which can  
be prepared by the novel synthesis method of the  
present invention as a result of the use of the  
extremely labile peptide-resin bond provided by the  
(orthochloro)trityl-resin bond. Thus, the particularly  
30 preferred acid-sensitive protecting groups which are  
uniquely useful in the novel method described above  
are, for example, trityl, t-Boc, t-butyl, Mtr, Pmc or  
formyl. The terms "acid-sensitive" and "sidechain  
protecting group" are used in accordance with their art



-39-

recognized definition, to the extent not inconsistent with the principles of the present invention.

The novel method of the present invention is also particularly well suited for the preparation of  
 5 analogs of a biologically active ligand having the Formula III, wherein  $X_1$  is Pyr (L or D), N-acyl-amino acid,  $\alpha$ -C-Alkyl-Pyr or N-acyl- $\alpha$ -C-alkyl-amino acid;  $X_2$ ,  $X_3$ ,  $X_4$ ,  $X_6$ ,  $X_7$  and  $X_8$  are independently natural, synthetic, protected or  $\alpha$ -C-alkyl- amino acid, or  $X_6$ - $X_7$   
 10 optionally contains a  $\gamma$ -lactam;  $X_9$  is imino acid or  $\alpha$ -C-alkyl-imino acid;  $X_{10}$  is -N(H)R wherein R is GlyNH<sub>2</sub>, azaGlyNH<sub>2</sub>, alkyl, alkenyl, haloalkyl, hydroxyalkyl, cycloalkyl or aryl, wherein each alkyl, alkenyl or aryl group has from 1 to 7 carbon atoms; and  $X_5$  is selected  
 15 according to Formulas I or II, as described above, with the proviso that the same compounds excluded from Formulas I and II are likewise excluded from the compounds according to Formula III.

The invention will be illustrated in greater  
 20 detail by the following specific examples. It is understood that these examples are given by way of illustration and are not meant to limit the disclosure of the claims to follow.

#### Example 1

25 Desensitization effects of angiotensin antagonists in the rat isolated uterus assay:

	<u>Analog</u>	<u>Recovery Time</u>
	[Sar <sup>1</sup> Ile <sup>8</sup> ]ANG II	180 min
	[Sar <sup>1</sup> Tyr(O-CH <sub>3</sub> ) <sup>4</sup> Ile <sup>8</sup> ]ANG II	20 min
30	[Sar <sup>1</sup> Phe(4'-F) <sup>4</sup> Ile <sup>8</sup> ]ANG II	20 min

-40-

Tissues were treated with the particular analog ( $10^{-5}$ M) for 2 min followed by washing out; time for recovery of the response to an ED50 dose of ANG II was determined.

### Example 2

- 5 Desensitization properties of GnRH agonists continuously superfused in goldfish pituitaries in vitro.

	<u>Peptide</u>	<u>Response (ng LH/ml)</u>				
		5 min	10 min	20 min	40 min	60 min
10	GnRH ( $10^{-6}$ M)	50	12	15	14	13
	[Tyr(Me) <sup>5</sup> ]GnRH	50	50	47	47	46
	( $5 \times 10^{-6}$ M)					

Methods were as described by Habibi, 1991 (Biology of Reproduction 44, 275-283).

- 15 More specifically, the relative responsiveness of goldfish pituitary fragments to various salmon and mammalian GnRH analogs were determined by using a superfusion system based on that described previously (Mackenzie et al., 1984; Chang et al., 1984; Marchant et al., 1987). Briefly, goldfish
- 20 pituitaries were removed, pars distalis were separated, and fragments were prepared ( $<0.5 \text{ mm}^2$ ) and placed between two  $100\text{-}\mu\text{l}$  layers of Cytodex carrier beads in a  $300\text{-}\mu\text{l}$  superfusion chamber (3 pituitary equivalents per
- 25 chamber). The fragments were superfused (5 ml/h) overnight (10-12 h) with Medium 199 containing Hanks' basic salts (Gibco Laboratories, Grand Island, N.Y.) 25 mM 4-(1-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 56 U/ml nystatin. Two hours prior to the
- 30 experiment, the medium was switched to Hanks' basic salts solution, supplemented with 25 mM HEPES buffer and 0.1% BSA (HBSS), and the flow rate was increased to 15 ml/h. Using a 3-way valve system, we exposed pituitary fragments to 2-min pulses of various

-41-

concentrations of various GnRH analogs ( $10^{-5}$ ,  $10^{-6}$ , and  $5 \times 10^{-5}$  M) every 60 min. All experiments involved running 4 or 8 columns simultaneously, with 5-min fractions collected automatically; samples were frozen at  $-25^{\circ}\text{C}$  until determination of GTH by a radioimmunoassay, as described previously (Peter et al., 1984).

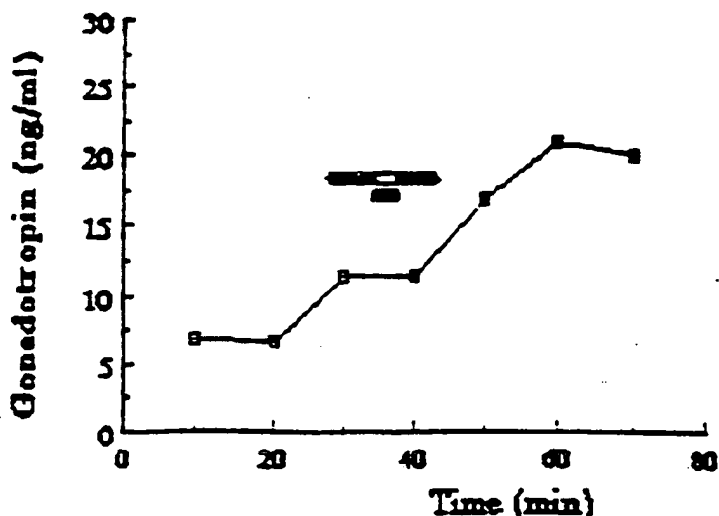
### Example 3

Desensitization properties of GnRH agonists after 2 min pulses every 20 min in superfused goldfish pituitaries in vitro according to the methods described in Example 2.

	Peptide	Pulse	Response (ng LH/ml)			
			#1	#2	#4	#7
15	GnRH ( $10^{-6}\text{M}$ )		60	14	13	10
	[Tyr(Me) <sup>5</sup> ]GnRH ( $5 \times 10^{-6}\text{M}$ )		36	32	33	38

### Example 4

Release of LH during superfusion of goldfish pituitaries in vitro induced by [Tyr(Me)<sup>5</sup>]GnRH in the absence (solid bar; 5 min) and presence (open bar; 3 min) of GnRH. Methods were as described by Habibi, 1991.



-42-

Example 5

Effect of subcutaneous injection of 1.5  $\mu$ g/injection of GnRH, [Tyr(Me)<sup>5</sup>]GnRH, or GnRH+[Tyr(Me)<sup>5</sup>]GnRH on serum LH levels in rats determined by radioimmunoassay

5

---

LH (Units/50  $\mu$ l serum)

---

	Time after injection	GnRH	[Tyr(Me) <sup>5</sup> ]GnRH	GnRH+[Tyr(Me) <sup>5</sup> ]GnRH
10	120 min	0.7	0	2.5
	150 min	0.1	0	1.9
	180 min	1.2	0	3.6
	225 min	-	0	3.3
	240 min	-	0	3.9

15

Rats were injected every 30 min with 1.5  $\mu$ g of GnRH, [Tyr(Me)<sup>5</sup>]GnRH, or GnRH+[Tyr(Me)<sup>5</sup>]GnRH. The values are corrected for basal levels (1.1 units/50  $\mu$ l) by subtraction.

20

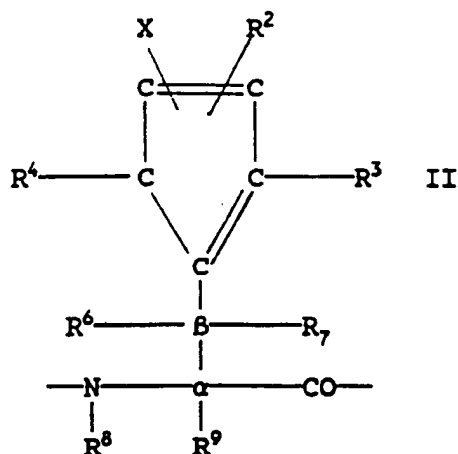
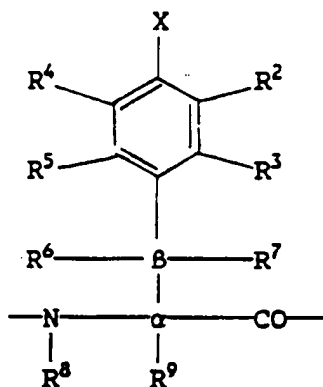
It will be noted that the analog of GnRH, [Tyr(Me)<sup>5</sup>]GnRH, administered alone was not effective due to the low dosage administered.

-43-

## CLAIMS

What is claimed is:

1. An analog of a biologically active ligand comprising at least one 6-membered ring replaced  
5 by Formula I or 5-membered ring replaced by Formula II:



wherein:

- X is selected from null, H, R<sup>1</sup>, -OR<sup>1</sup>, halide, -CN, -CHO, C(halide)<sub>3</sub>, -alk-OH, -alk-OR<sup>1</sup>, -alk-CO<sub>2</sub>H, -alk-CO<sub>2</sub>R<sup>1</sup>, -alk-SH, -alk-SR<sup>1</sup>, -alk-CONH<sub>2</sub>, -CO<sub>2</sub>H, -CO<sub>2</sub>R<sup>1</sup>,  
10 -COR<sup>1</sup>, -OCONH<sub>2</sub>, -OCH<sub>2</sub>OH, -OCH<sub>2</sub>OR<sup>1</sup>, -OCOR<sup>1</sup>, -N<sub>3</sub>, -N<sub>2</sub>, -NHCOR<sup>1</sup>, -NO<sub>2</sub>, -NH<sub>2</sub>, -NHR<sup>1</sup>, -NR<sup>1</sup><sub>2</sub>, -SO<sub>3</sub>H, -SO<sub>2</sub>R<sup>1</sup>, -SCOR<sup>1</sup>, -NCS, -SCSR<sup>1</sup>, -SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NHR<sup>1</sup>, -SO<sub>2</sub>NR<sup>1</sup><sub>2</sub>, -SO<sub>4</sub>H, -PO<sub>3</sub>H, -PO<sub>4</sub>H<sub>2</sub>, -SH, -SR<sup>1</sup>, -N=N-R<sup>1</sup>, =O, =NH, =N-R<sup>1</sup>, =N-OH, =N-OR<sup>1</sup>, -CONH<sub>2</sub>, -CONHR<sup>1</sup>, -CONR<sup>1</sup><sub>2</sub>, -NHSO<sub>2</sub>R<sup>1</sup>, -SiR<sup>1</sup><sub>3</sub>, tetrazole,  
15 imidazole, or substituted or unsubstituted phenyl, -alkylphenyl, -O-phenyl, -O-alkylphenyl, -O-benzyloxycarbonyl;

- R<sup>1</sup> is an alkyl of 1-7 carbon atoms, alkenyl or alkynyl of 2-7 carbon atoms or cycloalkyl of 3-7 carbon  
20 atoms, optionally halogenated in place of one or more hydrogen;

R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup> and R<sup>8</sup> are the same or different and are selected from X or -OH;

α is P, N, S, or C being in either the L- or

-44-

D-configuration and  $\beta$  may be deleted or extended by 1-2 carbon atoms, substituted or unsubstituted;

the nitrogen atom attached to the  $\alpha$  atom may be substituted or unsubstituted C, S, O or P and the  
5 carbon atom attached to the  $\alpha$  and the oxygen atoms may be substituted or unsubstituted N, S, O, P or C;

said aromatic ring of Formula I or said ring of Formula II may contain 0-4 N, S or O atoms;

said aromatic ring of Formula I or said ring  
10 of Formula II may be fused with a 6-membered ring which may also contain 0-4 N, S or O atoms and which may be substituted in the same manner as said aromatic ring of Formula I;

Formula I may be D-tyrosine and Formula II  
15 may be D-histidine; and wherein

$R^9$  is:

(i) X or OH, wherein X is not H; or

(ii) H, provided that the analog is not one of the following analogs based on mammalian GnRH:

20 (A) [D-Phe<sup>6</sup>]GnRH in which the hydroxyl group of tyrosine is protected with benzyl, acetyl, tosyl, benzoyl, t-butyl, tetrahydropyran-2-yl, trityl, 2,4-dichlorobenzyl, benzyloxycarbonyl or 2-bromobenzyloxycarbonyl;

25 (B) [Phe<sup>5</sup>]GnRH, [Ala<sup>4</sup>Phe<sup>5</sup>]GnRH and [Phe(N<sub>3</sub>)<sup>5</sup>]GnRH;

(C) [Des<sup>10</sup>Phe<sup>5</sup>-D-Ala<sup>6</sup>ProNHR<sup>9</sup>]GnRH in which position 2 is His, Tyr, Trp or Phe(4'-NH<sub>2</sub>), position 7 is Leu, Ile or Nle, and R is Et, Pr, CH<sub>2</sub>CH<sub>2</sub>OH or CHMe<sub>2</sub>;

30 (D) [Des<sup>10</sup>X<sup>5</sup>Pro-B<sup>9</sup>]GnRH in which position 3 is Trp, Phe or 3(1-naphthylalanine); X is His, Phe or Phe(F<sub>3</sub>); position 6 is D-Ala(Y) wherein Y is naphthyl, anthryl, fluorenyl, phenanthryl, biphenyl, benzhydryl, phenyl or cyclohexyl with 3 or more alkyl  
35 groups, perhydronaphthyl, adamantyl, perhydrobenzhydryl, phenyl, cyclohexyl, dicyclohexyl, heterocycle aryl, optionally N-alkylated alkylamine or

-45-

- cycloalkylamine, or  $\text{NHCH}[(\text{CH}_2)_n\text{NHC}(=\text{NR}_2)\text{R}_1]\text{CO}$  wherein:  $n$  is 1-5;  $\text{R}_1$  is alkyl or  $\text{NRR}_3$  wherein  $\text{R}$  is H or alkyl and  $\text{R}_3$  is H, alkyl, fluoroalkyl, cycloalkyl, phenyl, benzyl, alkyl-morpholino or  $(\text{CH}_2)_n(\text{R}_4)_2$  wherein  $n$  is 1-5 and  $\text{R}_4$  is alkyl;  $\text{R}_2$  is H or  $\text{R}_3$ ; or wherein  $\text{C}=\text{NR}_2\text{R}_1$  can be a ring; position 7 is Leu, Ile, MeLeu or Trp; position 8 is Arg, Gln, Tyr or Leu; and B is  $\text{GlyNH}_2$ ,  $\text{NHB}_2$  wherein  $\text{B}_2$  is alkyl, cycloalkyl or haloalkyl, and  $\text{NHCONHB}_3$  wherein  $\text{B}_3$  is H or alkyl;
- 10 (E) analogs containing a  $\gamma$ -lactam in the 6-7 position, in which position 1 is Pyr, N-acetyl, N-Pyr-imino acid, or  $(\text{C}_{3,7}$  cycloalkyl)acyl; positions 2 and 3 are aliphatic or aromatic amino acid; position 4 is Ser, Thr or Ala; position 5 is aromatic amino acid
- 15 His, Trp or Phe; position 8 is an amino acid with a basic sidechain; position 9 is imino acid or aliphatic amino acid; position 10 is  $\text{GlyNH}_2$ ,  $\text{AlaNH}_2$ ,  $\text{NHET}$ ,  $\text{NHPr}$ ,  $\text{NHCH}_2\text{CH}_2\text{OH}$ ; and
- (F) analogs in which position 5 is Phe,
- 20 Tyr(Me), N-alkyl-Phe, N-alkyl-Tyr(Phe) or N-alkyl-Tyr(Et), wherein the nitrogen atom of at least one of the amide bonds is alkylated.

2. The analog of Claim 1, wherein  $\text{R}^9$  is  $\text{R}_1$ .

3. The analog of Claim 2, wherein Formula I

25 comprises Tyr(alkyl), Phe(alkyl), or Phe(halide).

4. The analog of Claim 2, wherein Formula I comprises  $\alpha$ -C-alkyl-Tyr or Formula II comprises  $\alpha$ -C-alkyl-His.

5. The analog of Claim 1, wherein said

30 analog is selected from the group consisting of analogs of opiate peptides and analogs of Gonadotropin Releasing Hormone.

-46-

6. The analog of Claim 5, wherein the tyrosine residue of said analog of said opiate peptides or said analog of said Gonadotropin Releasing Hormone has been replaced by an analog according to Formula I.

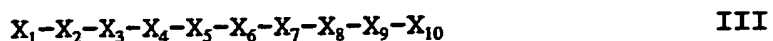
5           7. The analog of Claim 1, wherein said analog is further combined with at least one normally desensitizing analog of Gonadotropin Releasing Hormone or opiate peptide or synthetic analogs thereof.

10           8. A composition comprising an analog according to Claim 6 combined with at least one normally desensitizing analog of Gonadotropin Releasing Hormone or synthetic analog thereof.

15           9. The analog of Claim 1, wherein said analog includes a histidine residue modified according to Formula II.

10           10. The analog of Claim 1, wherein said analog comprises a non-peptidic ligand.

11. A compound having the Formula III:



20   wherein:

$X_1$  is Pyr, N-acyl-amino acid,  $\alpha$ -C-alkyl-Pyr, or N-acyl- $\alpha$ -C-alkyl-amino acid;

$X_2$ ,  $X_3$ ,  $X_4$ ,  $X_6$ ,  $X_7$  and  $X_8$  are independently selected from natural, synthetic, protected or  
25    $\alpha$ -C-alkyl- amino acids, wherein  $X_6$ - $X_7$  optionally contains a  $\gamma$ -lactam;

$X_9$  is an imino acid or  $\alpha$ -C-alkyl-imino acid;

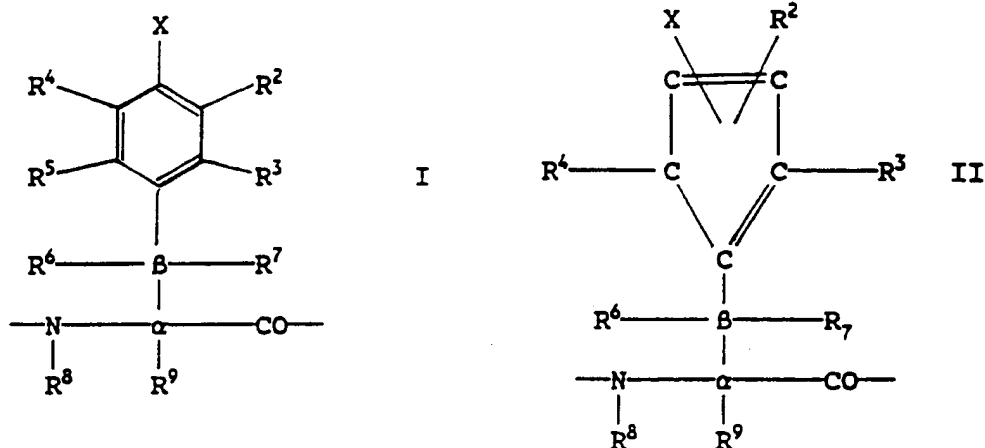
$X_{10}$  is -N(H)R wherein R is GlyNH<sub>2</sub>, azaGlyNH<sub>2</sub>, alkyl or alkenyl, cycloalkyl, haloalkyl, hydroxyalkyl



-47-

or aryl, wherein each alkyl, alkenyl or aryl group has from 1-7 carbon atoms; and

X<sub>5</sub> is selected from Formulas I or II:



wherein:

- 5 X is selected from null, H, R<sup>1</sup>, -OR<sup>1</sup>, halide, -CN, -CHO, C(halide)<sub>3</sub>, -alk-OH, -alk-OR<sup>1</sup>, -alk-CO<sub>2</sub>H, -alk-CO<sub>2</sub>R<sup>1</sup>, -alk-SH, -alk-SR<sup>1</sup>, -alk-CONH<sub>2</sub>, -CO<sub>2</sub>H, -CO<sub>2</sub>R<sup>1</sup>, -COR<sup>1</sup>, -OCONH<sub>2</sub>, -OCH<sub>2</sub>OH, -OCH<sub>2</sub>OR<sup>1</sup>, -OCOR<sup>1</sup>, -N<sub>3</sub>, -N<sub>2</sub>, -NHCOR<sup>1</sup>, -NO<sub>2</sub>, -NH<sub>2</sub>, -NHR<sup>1</sup>, -NR<sup>1</sup><sub>2</sub>, -SO<sub>3</sub>H, -SO<sub>2</sub>R<sup>1</sup>, -SCOR<sup>1</sup>,  
 10 -NCS, -SCSR<sup>1</sup>, -SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NHR<sup>1</sup>, -SO<sub>2</sub>NR<sup>1</sup><sub>2</sub>, -SO<sub>4</sub>H, -PO<sub>3</sub>H, -PO<sub>4</sub>H<sub>2</sub>, -SH, -SR<sup>1</sup>, -N=N-R<sup>1</sup>, =O, =NH, =N-R<sup>1</sup>, =N-OH, =N-OR<sup>1</sup>, -CONH<sub>2</sub>, -CONHR<sup>1</sup>, -CONR<sup>1</sup><sub>2</sub>, -NHSO<sub>2</sub>R<sup>1</sup>, -SiR<sup>1</sup><sub>3</sub>, tetrazole, imidazole, or substituted or unsubstituted phenyl, -alkylphenyl, -O-phenyl, -O-alkylphenyl,  
 15 -O-benzyloxycarbonyl;

R<sup>1</sup> is selected from alkyl of 1-7 carbon atoms, alkenyl or alkynyl of 2-7 carbon atoms or cycloalkyl of 3-7 carbon atoms, optionally halogenated in place of one or more hydrogen;

- 20 R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup> and R<sup>9</sup> are the same or different and are selected from X or -OH;

α is selected from P, N, S, or C being in either the L- or D-configuration and β is optionally deleted or extended by 1-2 carbon atoms, substituted or  
 25 unsubstituted;

-48-

the nitrogen atom attached to the  $\alpha$  atom is optionally substituted or unsubstituted C, S, O or P and the carbon atom attached to the  $\alpha$  and the oxygen atoms is optionally substituted or unsubstituted N, S, O, P or C;

said aromatic ring of Formula I or said ring of Formula II optionally has 0-4 N, S or O atoms;

said aromatic ring of Formula I or said ring of Formula II is optionally fused with a 6-membered ring which optionally has 0-4 N, S or O atoms and which is optionally substituted in the same manner as said aromatic ring of Formula I;

Formula I is optionally D-tyrosine and Formula II is optionally D-histidine; and

provided that when none of  $X_1 - X_9$  is an  $\alpha$ -C-alkyl- amino or imino acid, the compound is not:

(1) an antagonist analog of Gonadotropin Releasing Hormone obtained by substituting D-amino acids at positions 1, 2 or 3, or analogs which have position 2 deleted: or

(2) one of the following analogs based on mammalian GnRH:

(A) [D-Phe<sup>6</sup>]GnRH in which the hydroxyl group of tyrosine is protected with benzyl, acetyl, tosyl, benzoyl, t-butyl, tetrahydropyran-2-yl, trityl, 2,4-dichlorobenzyl, benzyloxycarbonyl or 2-bromobenzyloxycarbonyl;

(B) [Phe<sup>5</sup>]GnRH, [Ala<sup>4</sup>Phe<sup>5</sup>]GnRH and [Phe(N<sub>3</sub>)<sup>5</sup>]GnRH;

(C) [Des<sup>10</sup>Phe<sup>5</sup>-D-Ala<sup>6</sup>ProNHR<sup>9</sup>]GnRH in which position 2 is His, Tyr, Trp or Phe(4'-NH<sub>2</sub>), position 7 is Leu, Ile or Nle, and R is Et, Pr, CH<sub>2</sub>CH<sub>2</sub>OH or CHMe<sub>2</sub>;

(D) [Des<sup>10</sup>X<sup>5</sup>Pro-B<sup>9</sup>]GnRH in which position 3 is Trp, Phe or 3(1-naphthylalanine); X is His, Phe or Phe(F<sub>3</sub>); position 6 is D-Ala(Y) wherein Y is naphthyl, anthryl, fluorenyl, phenanthryl, biphenyl, benzhydryl, phenyl or cyclohexyl with 3 or more alkyl

-49-

groups, perhydronaphthyl, adamantyl, perhydrobenzhydryl, phenyl, cyclohexyl, dicyclohexyl, heterocycle aryl, optionally N-alkylated alkylamine or cycloalkylamine, or  $\text{NHCH}[(\text{CH}_2)_n\text{NHC}(=\text{NR}_2)\text{R}_1]\text{CO}$  wherein: n is 1-5;  $\text{R}_1$  is alkyl or  $\text{NRR}_3$  wherein R is H or alkyl and  $\text{R}_3$  is H, alkyl, fluoroalkyl, cycloalkyl, phenyl, benzyl, alkyl-morpholino or  $(\text{CH}_2)_n(\text{R}_4)_2$  wherein n is 1-5 and  $\text{R}_4$  is alkyl;  $\text{R}_2$  is H or  $\text{R}_3$ ; or wherein  $\text{C}=\text{NR}_2\text{R}_1$  can be a ring; position 7 is Leu, Ile, MeLeu or Trp; position 8 is Arg, Gln, Tyr or Leu; and B is  $\text{GlyNH}_2$ ,  $\text{NHB}_2$  wherein  $\text{B}_2$  is alkyl, cycloalkyl or haloalkyl, and  $\text{NHCONHB}_3$  wherein  $\text{B}_3$  is H or alkyl;

(E) analogs containing a  $\gamma$ -lactam in the 6-7 position, in which position 1 is Pyr, N-acetyl, N-Pyr-imino acid, or  $(\text{C}_{3-7}$  cycloalkyl)acyl; positions 2 and 3 are aliphatic or aromatic amino acid; position 4 is Ser, Thr or Ala; position 5 is aromatic amino acid His, Trp or Phe; position 8 is an amino acid with a basic sidechain; position 9 is imino acid or aliphatic amino acid; position 10 is  $\text{GlyNH}_2$ ,  $\text{AlaNH}_2$ ,  $\text{NHET}$ ,  $\text{NHPr}$ ,  $\text{NHCH}_2\text{CH}_2\text{OH}$ ; and

(F) analogs in which position 5 is Phe, Tyr(Me), N-alkyl-Phe, N-alkyl-Tyr(Phe) or N-alkyl-Tyr(Et), wherein the nitrogen atom of at least one of the amide bonds is alkylated.

12. The compound of Claim 11, wherein at least one of said  $\text{X}_1 - \text{X}_9$  of Formula III is an  $\alpha$ -C-alkyl- amino or imino acid.

13. The compound of Claim 12, wherein  $\text{X}_5$  of Formula III is Tyr(Me) or Tyr(Et).

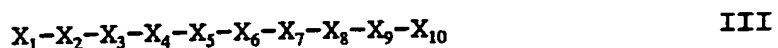
14. The compound of Claim 11, wherein  $\text{X}_5$  is  $\alpha$ -C-alkyl-Tyr or  $\alpha$ -C-alkyl-His.

-50-

15. The compound of Claim 11, wherein said compound includes a histidine residue modified according to Formula II.

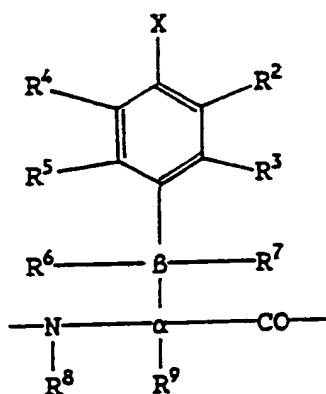
16. The compound of Claim 11, wherein said compound is an antagonist and is further combined with testosterone.

17. A compound having the Formula III:

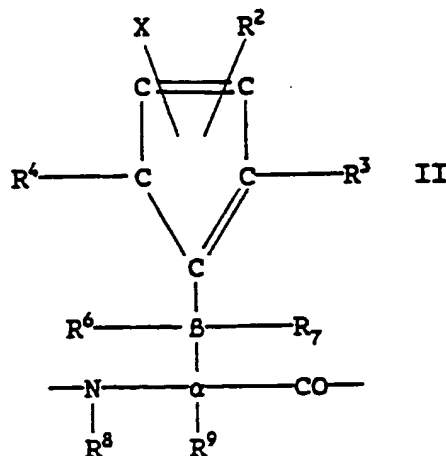


wherein:

- 10  $X_1$  is Pyr, N-acyl-amino acid,  $\alpha$ -C-alkyl-Pyr, or N-acyl- $\alpha$ -C-alkyl-amino acid;  
 $X_2$  and  $X_3$  are aromatic L-amino acids;  
 $X_4$ ,  $X_7$  and  $X_8$  are independently selected from natural, synthetic, protected or  $\alpha$ -C-alkyl-amino acids  
15  $X_6$  is a natural, synthetic, or protected D-amino acid, Gly or  $\alpha$ -C-alkyl-amino acid, wherein  $X_6$ - $X_7$  optionally contains a  $\gamma$ -lactam;  
 $X_9$  is an imino acid or  $\alpha$ -C-alkyl-imino acid;  
 $X_{10}$  is -N(H)R wherein R is GlyNH<sub>2</sub>, azaGlyNH<sub>2</sub>,  
20 alkyl or alkenyl, cycloalkyl, haloalkyl, hydroxyalkyl or aryl, wherein each alkyl, alkenyl or aryl group has from 1-7 carbon atoms; and  
 $X_5$  is selected from Formulas I or II:



I



II

-51-

wherein:

X is selected from null, H, R<sup>1</sup>, -OR<sup>1</sup>, halide, -CN, -CHO, C(halide)<sub>3</sub>, -alk-OH, -alk-OR<sup>1</sup>, -alk-CO<sub>2</sub>H, -alk-CO<sub>2</sub>R<sup>1</sup>, -alk-SH, -alk-SR<sup>1</sup>, -alk-CONH<sub>2</sub>, -CO<sub>2</sub>H, -CO<sub>2</sub>R<sup>1</sup>,  
 5 -COR<sup>1</sup>, -OCONH<sub>2</sub>, -OCH<sub>2</sub>OH, -OCH<sub>2</sub>OR<sup>1</sup>, -OCOR<sup>1</sup>, -N<sub>3</sub>, -N<sub>2</sub>, -NHCOR<sup>1</sup>, -NO<sub>2</sub>, -NH<sub>2</sub>, -NHR<sup>1</sup>, -NR<sup>1</sup><sub>2</sub>, -SO<sub>3</sub>H, -SO<sub>2</sub>R<sup>1</sup>, -SCOR<sup>1</sup>, -NCS, -SCSR<sup>1</sup>, -SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NHR<sup>1</sup>, -SO<sub>2</sub>NR<sup>1</sup><sub>2</sub>, -SO<sub>4</sub>H, -PO<sub>3</sub>H, -PO<sub>4</sub>H<sub>2</sub>, -SH, -SR<sup>1</sup>, -N=N-R<sup>1</sup>, =O, =NH, =N-R<sup>1</sup>, =N-OH, =N-OR<sup>1</sup>, -CONH<sub>2</sub>, -CONHR<sup>1</sup>, -CONR<sup>1</sup><sub>2</sub>, -NHSO<sub>2</sub>R<sup>1</sup>, -SiR<sup>1</sup><sub>3</sub>, tetrazole,  
 10 imidazole, or substituted or unsubstituted phenyl, -alkylphenyl, -O-phenyl, -O-alkylphenyl, -O-benzyloxycarbonyl;

R<sup>1</sup> is alkyl of 1-7 carbon atoms, alkenyl or alkynyl of 2-7 carbon atoms or cycloalkyl of 3-7 carbon  
 15 atoms, optionally halogenated in place of one or more hydrogen;

R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup> and R<sup>9</sup> are the same or different and are selected from X or -OH;

α is P, N, S, or C being in either the L- or  
 20 D-configuration and β is optionally deleted or extended by 1-2 carbon atoms, substituted or unsubstituted;

the nitrogen atom attached to the α atom is optionally substituted or unsubstituted C, S, O or P and the carbon atom attached to the α and the oxygen  
 25 atoms is optionally substituted or unsubstituted N, S, O, P or C;

said aromatic ring of Formula I or said ring of Formula II optionally has 0-4 N, S or O atoms;

said aromatic ring of Formula I or said ring  
 30 of Formula II is optionally fused with a 6-membered ring which optionally has 0-4 N, S or O atoms and which is optionally substituted in the same manner as said aromatic ring of Formula I;

Formula I is optionally D-tyrosine and  
 35 Formula II is optionally D-histidine; and

provided that when none of X<sub>1</sub>, or X<sub>4</sub> - X<sub>9</sub> is an α-C-alkyl- amino or imino acid, the compound is not:

-52-

(1) one of the following analogs based on mammalian GnRH:

(A) [D-Phe<sup>6</sup>]GnRH in which the hydroxyl group of tyrosine is protected with benzyl, acetyl, tosyl, benzoyl, t-butyl, tetrahydropyran-2-yl, trityl, 2,4-dichlorobenzyl, benzyloxycarbonyl or 2-bromobenzyloxycarbonyl;

(B) [Phe<sup>5</sup>]GnRH, [Ala<sup>4</sup>Phe<sup>5</sup>]GnRH and [Phe(N<sub>3</sub>)<sup>5</sup>]GnRH;

(C) [Des<sup>10</sup>Phe<sup>5</sup>-D-Ala<sup>6</sup>ProNHR<sup>9</sup>]GnRH in which position 2 is His, Tyr, Trp or Phe(4'-NH<sub>2</sub>), position 7 is Leu, Ile or Nle, and R is Et, Pr, CH<sub>2</sub>CH<sub>2</sub>OH or CHMe<sub>2</sub>;

(D) [Des<sup>10</sup>X<sup>5</sup>Pro-B<sup>9</sup>]GnRH in which position 3 is Trp, Phe or 3(1-naphthylalanine); X is His, Phe or Phe(F<sub>3</sub>); position 6 is D-Ala(Y) wherein Y is naphthyl, anthryl, fluorenyl, phenanthryl, biphenyl, benzhydryl, phenyl or cyclohexyl with 3 or more alkyl groups, perhydronaphthyl, adamantyl, perhydrobenzhydryl, phenyl, cyclohexyl, dicyclohexyl, heterocycle aryl, optionally N-alkylated alkylamine or cycloalkylamine, or NHCH[(CH<sub>2</sub>)<sub>n</sub>NHC(=NR<sub>2</sub>)R<sub>1</sub>]CO wherein: n is 1-5; R<sub>1</sub> is alkyl or NRR<sub>3</sub> wherein R is H or alkyl and R<sub>3</sub> is H, alkyl, fluoroalkyl, cycloalkyl, phenyl, benzyl, alkyl-morpholino or (CH<sub>2</sub>)<sub>n</sub>(R<sub>4</sub>)<sub>2</sub> wherein n is 1-5 and R<sub>4</sub> is alkyl; R<sub>2</sub> is H or R<sub>3</sub>; or wherein C=NR<sub>2</sub>R<sub>1</sub> can be a ring; position 7 is Leu, Ile, MeLeu or Trp; position 8 is Arg, Gln, Tyr or Leu; and B is GlyNH<sub>2</sub>, NHB<sub>2</sub> wherein B<sub>2</sub> is alkyl, cycloalkyl or haloalkyl, and NHCONHB<sub>3</sub> wherein B<sub>3</sub> is H or alkyl;

(E) analogs containing a γ-lactam in the 6-7 position, in which position 1 is Pyr, N-acetyl, N-Pyr-imino acid, or (C<sub>3-7</sub> cycloalkyl)acyl; positions 2 and 3 are aliphatic or aromatic amino acid; position 4 is Ser, Thr or Ala; position 5 is aromatic amino acid His, Trp or Phe; position 8 is an amino acid with a basic sidechain; position 9 is imino acid or aliphatic

-53-

amino acid; position 10 is GlyNH<sub>2</sub>, AlanH<sub>2</sub>, NH<sub>2</sub>Et, NHPr, NHCH<sub>2</sub>CH<sub>2</sub>OH; and

(F) analogs in which position 5 is Phe, Tyr(Me), N-alkyl-Phe, N-alkyl-Tyr(Phe) or N-alkyl-Tyr(Et), wherein the nitrogen atom of at least one of the amide bonds is alkylated.

18. The compound of Claim 17, wherein at least one of said X<sub>1</sub> and X<sub>4</sub> - X<sub>9</sub> of Formula III is an  $\alpha$ -C-alkyl-amino or imino acid.

10 19. The compound of Claim 18, wherein X<sub>5</sub> of Formula III is Tyr(Me) or Tyr(Et).

20. The compound of Claim 17, wherein X<sub>5</sub> of Formula III is Phe(halide), Phe(alkyl),  $\alpha$ -C-alkyl-Phe or  $\alpha$ -C-alkyl-Tyr(alkyl).

15 21. The compound of Claim 17, wherein X<sub>4</sub> of Formula III is Ser(Me), Thr(Me), Aib,  $\alpha$ -C-methyl-Ser(Me) or  $\alpha$ -C-methyl-Thr(Me).

22. The compound of Claim 17, wherein X<sub>6</sub> is  $\alpha$ -C-alkyl-amino acid.

20 23. The compound of Claim 17, wherein X<sub>7</sub> is  $\alpha$ -C-alkyl-amino acid.

24. The compound of Claim 17, wherein X<sub>8</sub> is  $\alpha$ -C-alkyl-amino acid.

25 25. The compound of Claim 17, wherein X<sub>6</sub> is a D-amino acid having a sidechain protecting group.

26. The compound of Claim 25, wherein said amino acid is His, Ser/Thr, Cys, Tyr, Arg, Lys, Orn/Lys, Asp/Glu, Asn/Gln or Trp.

-54-

27. The compound of Claim 25, wherein said protecting group of said amino acid of  $X_6$  is trityl, tosyl, dansyl, dinitrophenyl, benzyloxymethyl, t-butyl, t-Boc, acyl, Mtr, Pmc, xanthyl, formyl, or optionally substituted benzyl or benzyloxycarbonyl.

28. The compound of Claim 25, wherein  $X_6$  is D-His having the protecting group trityl, tosyl, dansyl, dinitrophenyl, benzyloxymethyl or optionally substituted benzyl; D-Ser/Thr having the protecting group trityl, t-butyl, acyl, optionally substituted benzyl or benzyloxycarbonyl; D-Cys having the protecting group trityl, t-butyl, tosyl, or optionally substituted benzyl; D-Tyr having the protecting group tosyl, trityl, acyl, t-butyl, optionally substituted benzyl or benzyloxycarbonyl; D-Arg having the protecting group Tos, Mtr or Pmc; D-Orn/Lys having the protecting group t-Boc, trityl or optionally substituted benzyloxycarbonyl; D-Asp/Glu having the protecting group t-butyl, trityl or optionally substituted benzyl; D-Asn/Gln having the protecting group trityl or xanthyl; D-Trp having the protecting group formyl or t-Boc.

29. The compound of Claim 25, wherein said protecting group is trityl, t-butyl, t-Boc, Mtr, Pmc or formyl.

30. The compound of Claim 17, wherein  $X_9$  is azetine-2-carboxyl.

31. The compound of Claim 17, wherein  $X_9$  is dehydroproline.

32. The compound of Claim 17, wherein  $X_9$  is nipecotyl.



-55-

33. A composition comprising an analog of Claim 17 in combination with at least one normally desensitizing analog of Gonadotropin Releasing Hormone or synthetic analog thereof.

5 34. A composition comprising an analog of Claim 29 in combination with at least one normally desensitizing analog of Gonadotropin Releasing Hormone or synthetic analog thereof.

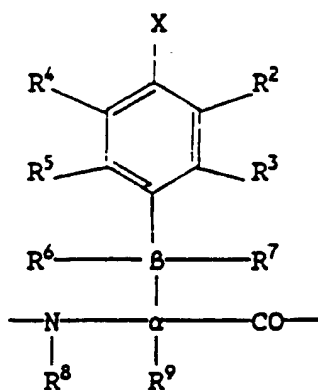
10 35. A opiate peptide comprising an N-terminal sequence of the Formula IV:



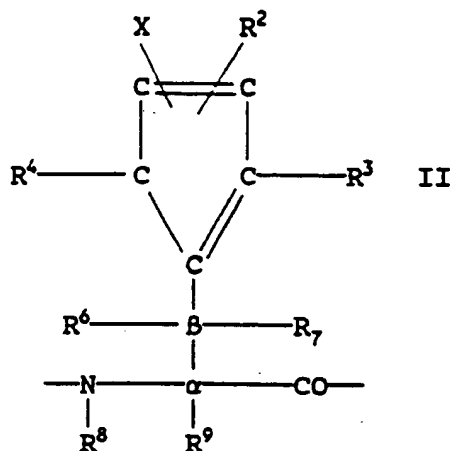
wherein

15  $X_2, X_3, X_4$  and  $X_5$  are independently selected from natural, synthetic, protected and  $\alpha$ -C-alkyl-amino acids wherein at least one of said  $X_2 - X_5$  is an  $\alpha$ -C-alkyl-amino acid;

$X_1$  is an amino acid residue selected according to Formulas I or II:



I



II

wherein:

20  $X$  is selected from null, H,  $R^1$ ,  $-OR^1$ , halide,  $-CN$ ,  $-CHO$ ,  $C(\text{halide})_3$ ,  $-alk-OH$ ,  $-alk-OR^1$ ,  $-alk-CO_2H$ ,  $-alk-CO_2R^1$ ,  $-alk-SH$ ,  $-alk-SR^1$ ,  $-alk-CONH_2$ ,  $-CO_2H$ ,  $-CO_2R^1$ ,  $-COR^1$ ,  $-OCONH_2$ ,  $-OCH_2OH$ ,  $-OCH_2OR^1$ ,  $-OCOR^1$ ,  $-N_3$ ,  $-N_2$ ,

-56-

-NHCOR<sup>1</sup>, -NO<sub>2</sub>, -NH<sub>2</sub>, -NHR<sup>1</sup>, -NR<sub>2</sub><sup>1</sup>, -SO<sub>3</sub>H, -SO<sub>2</sub>R<sup>1</sup>, -SCOR<sup>1</sup>,  
 -NCS, -SCSR<sup>1</sup>, -SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NHR<sup>1</sup>, -SO<sub>2</sub>NR<sub>2</sub><sup>1</sup>, -SO<sub>4</sub>H, -PO<sub>3</sub>H,  
 -PO<sub>4</sub>H<sub>2</sub>, -SH, -SR<sup>1</sup>, -N=N-R<sup>1</sup>, =O, =NH, =N-R<sup>1</sup>, =N-OH, =N-OR<sup>1</sup>,  
 -CONH<sub>2</sub>, -CONHR<sup>1</sup>, -CONR<sub>2</sub><sup>1</sup>, -NHSO<sub>2</sub>R<sup>1</sup>, -SiR<sub>3</sub><sup>1</sup>, tetrazole,  
 5 imidazole, or substituted or unsubstituted phenyl,  
 -alkylphenyl, -O-phenyl, -O-alkylphenyl,  
 -O-benzyloxycarbonyl;

R<sup>1</sup> is an alkyl of 1-7 carbon atoms, an alkenyl  
 or alkynyl of 2-7 carbon atoms or cycloalkyl of  
 10 3-7 carbon atoms, optionally halogenated in place of  
 one or more hydrogen;

R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup> and R<sup>9</sup> are the same or  
 different and are X or -OH;

α is P, N, S, or C being in either the L- or  
 15 D-configuration and β is optionally deleted or extended  
 by 1-2 carbon atoms, substituted or unsubstituted;

the nitrogen atom attached to the α atom is  
 optionally substituted or unsubstituted C, S, O or P  
 and the carbon atom attached to the α and the oxygen  
 20 atoms is optionally substituted or unsubstituted N, S,  
 O, P or C;

said aromatic ring of Formula I or said ring  
 of Formula II optionally has 0-4 N, S or O atoms;

said aromatic ring of Formula I or said ring  
 25 of Formula II is optionally fused with a 6-membered  
 ring which optionally has 0-4 N, S or O atoms and which  
 is optionally substituted in the same manner as said  
 aromatic ring of Formula I; and wherein

Formula I is optionally D-tyrosine and  
 30 Formula II is optionally D-histidine.

36. The compound of Claim 35, wherein X<sub>1</sub> of  
 Formula IV is Tyr(Me) or Tyr(Et).

37. The compound of Claim 35, wherein X<sub>1</sub> of  
 Formula IV is Phe(halide), Phe(alkyl), α-C-alkyl-Phe or  
 35 α-C-alkyl-Tyr(alkyl).

-57-

38. The compound of Claim 35, wherein at least one of  $X_2 - X_5$  is an  $\alpha$ -C-alkyl-amino acid.

39. The compound of Claim 36, wherein at least one of  $X_2 - X_5$  is an amino acid having a sidechain  
5 protecting group.

40. The compound of Claim 39, wherein said amino acid is His, Ser/Thr, Cys, Tyr, Arg, Lys, Orn/Lys, Asp/Glu, Asn/Gln or Trp.

41. The compound of Claim 39, wherein said  
10 protecting group of said amino acid of  $X_4$  or  $X_5$  is trityl, tosyl, dansyl, dinitrophenyl, benzyloxymethyl, t-butyl, t-Boc, acyl, Mtr, Pmc, xanthyl, formyl, or optionally substituted benzyl or benzyloxycarbonyl.

42. The compound of Claim 39, wherein  $X_4$  or  
15  $X_5$  is His having the protecting group trityl, tosyl, dansyl, dinitrophenyl, benzyloxymethyl or optionally substituted benzyl; Ser/Thr having the protecting group trityl, t-butyl, acyl, optionally substituted benzyl or benzyloxycarbonyl; Cys having the protecting group  
20 trityl, t-butyl, tosyl, or optionally substituted benzyl; Tyr having the protecting group tosyl, trityl, acyl, t-butyl, optionally substituted benzyl or benzyloxycarbonyl; Arg having the protecting group Tos, Mtr or Pmc; Lys having the protecting group t-Boc,  
25 trityl or optionally substituted benzyloxycarbonyl; Orn/Lys having the protecting group t-Boc, trityl or optionally substituted benzyloxycarbonyl; Asp/Glu having the protecting group t-butyl, trityl or optionally substituted benzyl; Asn/Gln having the  
30 protecting group trityl or xanthyl; Trp having the protecting group formyl or t-Boc.

-58-

43. The compound of Claim 39, wherein said protecting group is trityl, t-butyl, t-Boc, Mtr, Pmc or formyl.

5 44. The compound of Claim 35, wherein said compound includes a histidine residue modified according to Formula II.

45. A composition comprising an analog according to Claim 35 in combination with at least one normally desensitizing compound of an opiate peptide or  
10 synthetic analog thereof.

46. A composition comprising an analog according to Claim 38 in combination with at least one normally desensitizing compound of an opiate peptide or synthetic analog thereof.

15 47. A method for synthesizing a peptide while retaining an acid-sensitive protecting group comprising employing a chloro-(orthochloro)trityl resin as the means for attaching the terminal amino acid thus allowing for the use of mild acid conditions for the  
20 cleavage of the peptide-resin bond.

48. The method of claim 47, wherein the peptide synthesized by said method is according to Claim 1.

25 49. The method of claim 47, wherein the peptide synthesized by said method is according to Claim 11.

50. The method of claim 47, wherein the peptide synthesized by said method is according to Claim 17.

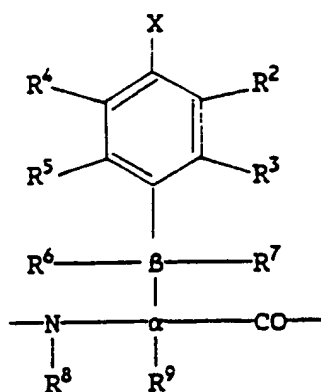
-59-

51. The method of claim 47, wherein the peptide synthesized by said method is according to Claim 29.

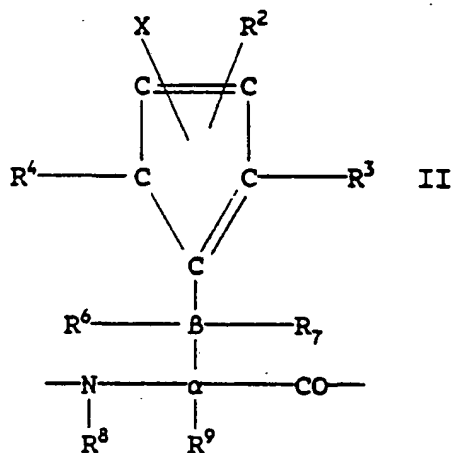
52. The method of claim 47, wherein the peptide synthesized by said method is according to Claim 35.

53. The method of claim 47, wherein the peptide synthesized by said method is according to Claim 43.

54. Method for selecting non-desensitizing analogs of a biologically active ligand containing at least one 6-membered ring or 5-membered ring, comprising selecting an analog of a biologically active ligand containing a 6-membered ring which has been replaced by the the Formula I or selecting a biologically active ligand containing a 5-membered ring which has been replaced by the Formula II:



I



II

wherein:

X is selected from null, H,  $R^1$ ,  $-OR^1$ , halide,  $-CN$ ,  $-CHO$ ,  $C(\text{halide})_3$ ,  $-alk-OH$ ,  $-alk-OR^1$ ,  $-alk-CO_2H$ ,  $-alk-CO_2R^1$ ,  $-alk-SH$ ,  $-alk-SR^1$ ,  $-alk-CONH_2$ ,  $-CO_2H$ ,  $-CO_2R^1$ ,  $-COR^1$ ,  $-OCONH_2$ ,  $-OCH_2OH$ ,  $-OCH_2OR^1$ ,  $-OCOR^1$ ,  $-N_3$ ,  $-N_2$ ,

-60-

-NHCOR<sup>1</sup>, -NO<sub>2</sub>, -NH<sub>2</sub>, -NHR<sup>1</sup>, -NR<sub>2</sub><sup>1</sup>, -SO<sub>3</sub>H, -SO<sub>2</sub>R<sup>1</sup>, -SCOR<sup>1</sup>,  
-NCS, -SCSR<sup>1</sup>, -SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NHR<sup>1</sup>, -SO<sub>2</sub>NR<sub>2</sub><sup>1</sup>, -SO<sub>4</sub>H, -PO<sub>3</sub>H,  
-PO<sub>4</sub>H<sub>2</sub>, -SH, -SR<sup>1</sup>, -N=N-R<sup>1</sup>, =O, =NH, =N-R<sup>1</sup>, =N-OH, =N-OR<sup>1</sup>,  
-CONH<sub>2</sub>, -CONHR<sup>1</sup>, -CONR<sub>2</sub><sup>1</sup>, -NHSO<sub>2</sub>R<sup>1</sup>, -SiR<sub>3</sub><sup>1</sup>, tetrazole,  
5 imidazole, or substituted or unsubstituted phenyl,  
-alkylphenyl, -O-phenyl, -O-alkylphenyl,  
-O-benzyloxycarbonyl;

R<sup>1</sup> is an alkyl of 1-7 carbon atoms, an alkenyl  
or alkynyl of 2-7 carbon atoms or cycloalkyl of  
10 3-7 carbon atoms, optionally halogenated in place of  
one or more hydrogen;

R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup> and R<sup>9</sup> are groups  
independently selected from X, alkyl or -OH;

α is P, N, S, or C being in either the L- or  
15 D-configuration;

β is optionally deleted or extended by 1-2  
carbon atoms, substituted or unsubstituted;

the nitrogen atom attached to α is optionally  
substituted or unsubstituted C, S, O or P;

20 the carbon atom attached to α and oxygen is  
optionally substituted or unsubstituted N, S, O, P or  
C;

the aromatic ring of Formula I or the ring of  
Formula II optionally has 0-4 N, S or O atoms;

25 the aromatic ring of Formula I or ring of  
Formula II is optionally fused with a 6-membered ring  
which optionally has 0-4 N, S or O atoms which is  
optionally substituted in the same manner as the  
aromatic ring of Formula I;

30 the ring of Formula I is optionally  
D-tyrosine or the ring of Formula II is optionally D-  
histidine; and

wherein the analog is selected so that the  
resulting biologically active ligand is such that any  
35 subsequent interaction of said ligand with a  
biologically active receptor does not substantially  
attenuate said receptor.

-61-

55. The method of Claim 54, wherein R<sup>9</sup> is:

(i) X or OH, wherein X is not H; or

(ii) H, provided that the analog is not one of the following analogs based on mammalian GnRH:

5 (A) [D-Phe<sup>6</sup>]GnRH in which the hydroxyl group of tyrosine is protected with benzyl, acetyl, tosyl, benzoyl, t-butyl, tetrahydropyran-2-yl, trityl, 2,4-dichlorobenzyl, benzyloxycarbonyl or 2-bromobenzyloxycarbonyl;

10 (B) [Phe<sup>5</sup>]GnRH, [Ala<sup>4</sup>Phe<sup>5</sup>]GnRH and [Phe(N<sub>3</sub>)<sup>5</sup>]GnRH;

(C) [Des<sup>10</sup>Phe<sup>5</sup>-D-Ala<sup>6</sup>ProNHR<sup>9</sup>]GnRH in which position 2 is His, Tyr, Trp or Phe(4'-NH<sub>2</sub>), position 7 is Leu, Ile or Nle, and R is Et, Pr, CH<sub>2</sub>CH<sub>2</sub>OH or CHMe<sub>2</sub>;

15 (D) [Des<sup>10</sup>X<sup>5</sup>Pro-B<sup>9</sup>]GnRH in which position 3 is Trp, Phe or 3(1-naphthylalanine); X is His, Phe or Phe(F<sub>3</sub>); position 6 is D-Ala(Y) wherein Y is naphthyl, anthryl, fluorenyl, phenanthryl, biphenyl, benzhydryl, phenyl or cyclohexyl with 3 or more alkyl groups, perhydronaphthyl, adamantyl, perhydrobenzhydryl, phenyl, cyclohexyl, dicyclohexyl, heterocycle aryl, optionally N-alkylated alkylamine or cycloalkylamine, or NHCH[(CH<sub>2</sub>)<sub>n</sub>NHC(=NR<sub>2</sub>)R<sub>1</sub>]CO wherein: n is 1-5; R<sub>1</sub> is alkyl or NRR<sub>3</sub> wherein R is H or alkyl and R<sub>3</sub> is H, alkyl, fluoroalkyl, cycloalkyl, phenyl, benzyl, alkyl-morpholino or (CH<sub>2</sub>)<sub>n</sub>(R<sub>4</sub>)<sub>2</sub> wherein n is 1-5 and R<sub>4</sub> is alkyl; R<sub>2</sub> is H or R<sub>3</sub>; or wherein C=NR<sub>2</sub>R<sub>1</sub> can be a ring; position 7 is Leu, Ile, MeLeu or Trp; position 8 is Arg, Gln, Tyr or Leu; and B is selected from GlyNH<sub>2</sub>, NHB<sub>2</sub> wherein B<sub>2</sub> is alkyl, cycloalkyl or haloalkyl, and NHCONHB<sub>3</sub> wherein B<sub>3</sub> is H or alkyl;

20 (E) analogs containing a γ-lactam in the 6-7 position, in which position 1 is Pyr, N-acetyl, N-Pyr-imino acid, or (C<sub>3-7</sub> cycloalkyl)acyl; positions 2 and 3 are aliphatic or aromatic amino acid; position 4 is Ser, Thr or Ala; position 5 is aromatic

-62-

amino acid His, Trp or Phe; position 8 is an amino acid with a basic sidechain; position 9 is imino acid or aliphatic amino acid; position 10 is GlyNH<sub>2</sub>, AlaNH<sub>2</sub>, NH<sub>2</sub>Et, NHPr, NHCH<sub>2</sub>CH<sub>2</sub>OH; and

- 5 (F) analogs in which position 5 is Phe, Tyr(Me), N-alkyl-Phe, N-alkyl-Tyr(Phe) or N-alkyl-Tyr(Et), wherein the nitrogen atom of at least one of the amide bonds is alkylated.

- 10 56. The method of Claim 54, wherein said analog of a biologically active ligand is selected from the group consisting of analogs of opiate peptides and analogs of Gonadotropin Releasing Hormone.

- 15 57. The method of Claim 56, wherein at least one of said analogs of Gonadotropin Releasing Hormone is used alone or in combination with at least one normally desensitizing Gonadotropin Releasing Hormone or synthetic analog thereof.

- 20 58. The method of Claim 57, further comprising the use of domperidone in combination with said at least one analog of Gonadotropin Releasing Hormone.

- 25 59. The method of Claim 56, wherein at least one of said analogs of an opiate peptide is used alone or in combination with at least one normally desensitizing analog of an opiate ligand or synthetic analog thereof.

- 30 60. The method of Claim 56, wherein the tyrosine residue of said analog of said opiate peptides or said analog of said Gonadotropin Releasing Hormone has been selected according to Formula I.



-63-

61. The method of Claim 54, wherein lifetime fluorescence and/or NMR spectroscopy on said biologically active ligand in a suitable receptor-simulating environment is used to determine the non-  
5 desensitizing nature of said ligand.

62. The method of Claim 54, wherein said analog contains at least one 5-membered ring and is selected to contain a substituted or unsubstituted histidine residue modified according to Formula II.

10 63. The method of Claim 56, wherein the tyrosine of said Gonadotropin Releasing Hormone has been substituted with histidine modified according to Formula II and wherein said Gonadotropin Releasing Hormone is mammalian, salmon or chicken I Gonadotropin  
15 Releasing Hormone.

64. The method of Claim 54, wherein said analog of a biologically active ligand comprises a non-peptidic ligand.

20 65. The method of Claim 64, wherein said non-peptidic ligand is selected from the group consisting of substituted or unsubstituted steroids, substituted or unsubstituted catecholamines and substituted or unsubstituted nonpeptide opiate ligands.

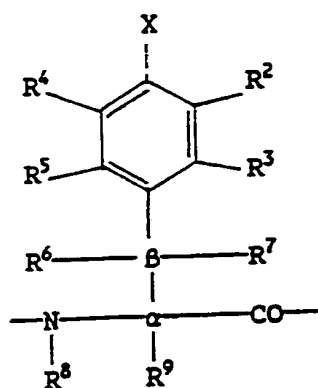
25 66. The method of Claim 56, wherein said analog of said biologically active ligand is an antagonist and is used in combination with testosterone.

30 67. The method of Claim 57, wherein said analog of said biologically active ligand is an antagonist and is used in combination with testosterone.

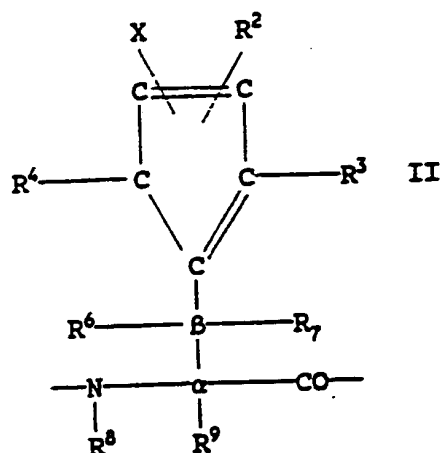
-64-

68. Method of treating a patient in a non-desensitizing fashion with a non-desensitizing analog of a biologically active ligand containing at least one 6-membered aromatic ring or 5-membered ring, comprising:

- (A) selecting a non-desensitizing analog of a biologically active ligand containing a 6-membered ring which has been replaced by the Formula I or selecting an analog of a biologically active ligand containing a 5-membered ring which has been replaced by the Formula II:



I



II

wherein:

- X is selected from null, H, R<sup>1</sup>, -OR<sup>1</sup>, halide, -CN, -CHO, C(halide)<sub>3</sub>, -alk-OH, -alk-OR<sup>1</sup>, -alk-CO<sub>2</sub>H, -alk-CO<sub>2</sub>R<sup>1</sup>, -alk-SH, -alk-SR<sup>1</sup>, -alk-CONH<sub>2</sub>, -CO<sub>2</sub>H, -CO<sub>2</sub>R<sup>1</sup>, -COR<sup>1</sup>, -OCONH<sub>2</sub>, -OCH<sub>2</sub>OH, -OCH<sub>2</sub>OR<sup>1</sup>, -OCOR<sup>1</sup>, -N<sub>3</sub>, -N<sub>2</sub>, -NHCOR<sup>1</sup>, -NO<sub>2</sub>, -NH<sub>2</sub>, -NHR<sup>1</sup>, -NR<sup>1</sup><sub>2</sub>, -SO<sub>3</sub>H, -SO<sub>2</sub>R<sup>1</sup>, -SCOR<sup>1</sup>, -NCS, -SCSR<sup>1</sup>, -SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NHR<sup>1</sup>, -SO<sub>2</sub>NR<sup>1</sup><sub>2</sub>, -SO<sub>4</sub>H, -PO<sub>3</sub>H, -PO<sub>4</sub>H<sub>2</sub>, -SH, -SR<sup>1</sup>, -N=N-R<sup>1</sup>, =O, =NH, =N-R<sup>1</sup>, =N-OH, =N-OR<sup>1</sup>, -CONH<sub>2</sub>, -CONHR<sup>1</sup>, -CONR<sup>1</sup><sub>2</sub>, -NHSO<sub>2</sub>R<sup>1</sup>, -SiR<sup>1</sup><sub>3</sub>, tetrazole, imidazole, or substituted or unsubstituted phenyl, -alkylphenyl, -O-phenyl, -O-alkylphenyl, -O-benzyloxycarbonyl;

- R<sup>1</sup> is an alkyl of 1-7 carbon atoms, an alkenyl or alkynyl of 2-7 carbon atoms or cycloalkyl of

-65-

3-7 carbon atoms, optionally halogenated in place of one or more hydrogen;

$R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^8$  and  $R^9$  are X, alkyl or -OH;

5            $\alpha$  is P, N, S, or C being in either the L- or D-configuration;

$\beta$  is optionally deleted or extended by 1-2 carbon atoms, substituted or unsubstituted;

10           the nitrogen atom attached to  $\alpha$  is optionally substituted or unsubstituted C, S, O or P;

            the carbon atom attached to  $\alpha$  and oxygen is optionally substituted or unsubstituted N, S, O, P or C;

15           the aromatic ring of Formula I or ring of Formula II optionally has 0-4 N, S or O atoms;

            the aromatic ring of Formula I or ring of Formula II is optionally fused with a 6-membered ring which optionally has 0-4 N, S or O atoms which can be substituted in the same manner as the aromatic ring of  
20   Formula I;

            the ring of Formula I is optionally D-tyrosine or the ring of Formula II is optionally D-histidine; and

25           (B) administering to a patient said analog of a biologically active ligand selected in step (A) as a composition comprising:

            (i) a pharmaceutically effective amount of said analog of a biologically active ligand selected in step (A); and

30           (ii) a pharmaceutically acceptable carrier for said analog of a biologically active ligand.

69. The method of Claim 68, wherein  $R^9$  is:

35           (i) X or OH, wherein X is not H; or  
            (ii) H, provided that the analog is not one of the following analogs based on mammalian GnRH:

-66-

- (A) [D-Phe<sup>6</sup>]GnRH in which the hydroxyl group of tyrosine is protected with benzyl, acetyl, tosyl, benzoyl, t-butyl, tetrahydropyran-2-yl, trityl, 2,4-dichlorobenzyl, benzyloxycarbonyl or 2-bromobenzyloxycarbonyl;
- (B) [Phe<sup>5</sup>]GnRH, [Ala<sup>4</sup>Phe<sup>5</sup>]GnRH and [Phe(N<sub>3</sub>)<sup>5</sup>]GnRH;
- (C) [Des<sup>10</sup>Phe<sup>5</sup>-D-Ala<sup>6</sup>ProNHR<sup>9</sup>]GnRH in which position 2 is His, Tyr, Trp or Phe(4'-NH<sub>2</sub>), position 7 is Leu, Ile or Nle, and R is Et, Pr, CH<sub>2</sub>CH<sub>2</sub>OH or CHMe<sub>2</sub>;
- (D) [Des<sup>10</sup>X<sup>6</sup>Pro-B<sup>9</sup>]GnRH in which position 3 is Trp, Phe or 3(1-naphthylalanine); X is His, Phe or Phe(F<sub>3</sub>); position 6 is D-Ala(Y) wherein Y is naphthyl, anthryl, fluorenyl, phenanthryl, biphenyl, benzhydryl, phenyl or cyclohexyl with 3 or more alkyl groups, perhydronaphthyl, adamantyl, perhydrobenzhydryl, phenyl, cyclohexyl, dicyclohexyl, heterocycle aryl, optionally N-alkylated alkylamine or cycloalkylamine, or NHCH[(CH<sub>2</sub>)<sub>n</sub>NHC(=NR<sub>2</sub>)R<sub>1</sub>]CO wherein: n is 1-5; R<sub>1</sub> is alkyl or NRR<sub>3</sub> wherein R is H or alkyl and R<sub>3</sub> is H, alkyl, fluoroalkyl, cycloalkyl, phenyl, benzyl, alkyl-morpholino or (CH<sub>2</sub>)<sub>n</sub>(R<sub>4</sub>)<sub>2</sub> wherein n is 1-5 and R<sub>4</sub> is alkyl; R<sub>2</sub> is H or R<sub>3</sub>; or wherein C=NR<sub>2</sub>R<sub>1</sub> can be a ring; position 7 is Leu, Ile, MeLeu or Trp; position 8 is Arg, Gln, Tyr or Leu; and B is GlyNH<sub>2</sub>, NHB<sub>2</sub> wherein B<sub>2</sub> is alkyl, cycloalkyl or haloalkyl, and NHCONHB<sub>3</sub> wherein B<sub>3</sub> is H or alkyl;
- (E) analogs containing a γ-lactam in the 6-7 position, in which position 1 is Pyr, N-acetyl, N-Pyr-imino acid, or (C<sub>3-7</sub> cycloalkyl)acyl; positions 2 and 3 are aliphatic or aromatic amino acid; position 4 is Ser, Thr or Ala; position 5 is aromatic amino acid His, Trp or Phe; position 8 is an amino acid with a basic sidechain; position 9 is imino acid or aliphatic amino acid; position 10 is GlyNH<sub>2</sub>, AlanH<sub>2</sub>, NH<sub>2</sub>Et, NH<sub>2</sub>Pr, NHCH<sub>2</sub>CH<sub>2</sub>OH; and

-67-

(F) analogs in which position 5 is Phe, Tyr(Me), N-alkyl-Phe, N-alkyl-Tyr(Phe) or N-alkyl-Tyr(Et), wherein the nitrogen atom of at least one of the amide bonds is alkylated.

5           70. The method of Claim 68, wherein said analog of a biologically active ligand is selected from the group consisting of analogs of opiate peptides and analogs of Gonadotropin Releasing Hormone.

10           71. The method of Claim 70, wherein the tyrosine of said analog of said opiate peptides or said Gonadotropin Releasing Hormone has been selectively modified according to Formula I.

15           72. The method of Claim 71, wherein at least one of said analogs of Gonadotropin Releasing Hormone is used alone or in combination with at least one normally desensitizing Gonadotropin Releasing Hormone or synthetic analog thereof.

20           73. The method of Claim 72, further comprising the use of domperidone in combination with said at least one analog of Gonadotropin Releasing Hormone.

25           74. The method of Claim 70, wherein at least one of said analogs of an opiate peptide is used alone or in combination with at least one normally desensitizing analog of an opiate ligand or synthetic analog thereof.

30           75. The method of Claim 68, wherein lifetime fluorescence and/or NMR spectroscopy on said biologically active ligand in a suitable receptor-simulating environment is used to determine the non-desensitizing nature of said ligand.

-68-

76. The method of Claim 68, wherein said analog containing at least one 5-membered ring is selected as a substituted or unsubstituted histidine residue selectively modified according to Formula II.

5           77. The method of Claim 68, wherein the tyrosine of said Gonadotropin Releasing Hormone has been substituted with histidine selectively modified according to Formula II and wherein said Gonadotropin Releasing Hormone is mammalian, salmon or chicken I  
10 Gonadotropin Releasing Hormone.

78. The method of Claim 68, wherein said analog of a biologically active ligand comprises a non-peptidic ligand.

15           79. The method of Claim 78, wherein said non-peptidic ligand is selected from the group consisting of substituted or unsubstituted steroids, substituted or unsubstituted catecholamines and substituted or unsubstituted nonpeptide opiate ligands.

20           80. The method of Claim 70, wherein said analog of said biologically active ligand is an antagonist and is used in combination with testosterone.

25           81. The method of Claim 71, wherein said analog of said biologically active ligand is an antagonist and is used in combination with testosterone.

**This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record.**

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☒ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☒ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☒ **OTHER:** \_\_\_\_\_

## **IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**

**THIS PAGE BLANK (USPTO)**